INHERITANCE OF STIGMA TYPE IN MATERIALS DERIVED FROM HYBRIDIZATION OF *Phaseolus vulgaris* L. AND *Phaseolus coccineus* L.

BY

RICHARD MICHAEL MANSHARDT

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In Memory of

John W. Wilhelms, Ph.D.,

farmer's son, athlete, and scholar,

for whom this token of admiration

comes too late.

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Richard Michael Manshardt

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The possibility of introducing the outcrossing flower structure of the scarlet runner bean, *Phaseolus coccineus* L., into germplasm of the self-pollinating common bean, *P. vulgaris* L., was investigated as a means of producing outcrossing common beans suitable for use in population improvement schemes. The chief determinant of outcrossing ability in *P. coccineus* is the extrorse stigma position of the stigma. The goal of this study was to transfer the extrorse stigma type from *P. coccineus* into *P. vulgaris* germplasm through interspecific hybridization.

Lengths of internal surfaces (Int) and external surfaces (Ext) of stigmas were measured microscopically. The Ext/Int ratio was used as an index of stigma shape.

The major *P. vulgaris* cultivars employed were 'Swiss', 'Harvester', 'Sprite' and 'Light Red Kidney', while the *P. coccineus* materials used were 'Hammond's Dwarf White', PI 273666, PI 311819, PI 312009 and PI 321088.

Five interspecific F_2 populations were produced, and segregants with Ext/Int stigma ratios greater than 0.70 from these and advanced inbred lines were backcrossed to P. vulgaris. Stigma measurement distributions in F_1, F_2, backcrosses of F_1's to both parental species, and F_3 generations indicated that stigma shape is a quantitative character with a polygenic mode of inheritance. The only exception was an F_2 population that showed evidence of major gene segregation for introrse terminal and extrorse stigma classes, suggesting a 2-gene system with dominant epistasis. However, the latter may have been attributable to a qualitative, rather than a quantitative, classification of stigma types and to a small sample size. Correlation between mean stigma measurements of F_3 progenies and stigma measurements of F_2 parents was high. Narrow sense heritability estimates for stigma measurements, calculated from correlation coefficients, averaged 60%.

Inbreeding produced nearly homozygous F_3, F_4, and F_5 progenies, the means of which had Ext/Int ratios that formed a continuous distribution. The failure of homozygous genotypes to cluster indicates that a large number of genes govern stigma shape. Fertility of the inbreds declined with each generation of inbreeding. Generally speaking, it was not possible to progress beyond the F_4 generation due to self-sterility.

Segregants with Ext/Int ratios greater than 0.70 in F_2, F_3, and F_4 generations were backcrossed to P. vulgaris. In the resultant BC_1-F_2 and BC_2-F_2 generations, only about 1% of the segregating population recovered the parental phenotypes with Ext/Int greater than 0.70. The low frequency of terminal stigma types in BC-F_2 populations argues for polygenic control of stigma shape.

Results of a reciprocal testcross showed that failure to recover a greater proportion of terminal and extrorse stigma types in BC-F_2 populations was not due to selective elimination of stigma alleles of *P. coccineus*. Furthermore, the number of parental segregates recovered in the testcross progenies indicated that a minimum of 5 to 7 genes was segregating for stigma shape in these progenies.

Backcrossing to *P. vulgaris* did not improve the general level of fertility above that of the interspecific F_2 generation. There was no improvement in fertility between BC_1-F_2 and BC_2-F_2 generations. Plants with Ext/Int ratios greater than 0.70 seemed more sterile than those with Ext/Int ratios less than 0.70. Sterility factors may be linked to a block of *P. coccineus* stigma alleles in an inverted chromosome segment, or alternatively, sterility may be a pleiotropic effect of P. coccineus alleles in interspecific hybrids.

The sterility accompanying stigma alleles of *P. coccineus* and the large number of genes controlling stigma inheritance make it doubtful that stigma alleles of *P. coccineus* will be employed in *P. vulgaris* germplasm to provide an outcrossing mechanism suitable for population improvement breeding schemes.

INTRODUCTION

The common bean, Phaseolus vulgaris L., is planted over a larger area, and has a greater overall production, than any other grain legume consumed directly as human food. About 85% of the world crop is produced in developing nations (FAO, 1978), where it provides a relatively inexpensive source of protein. Furthermore, bean proteins contain adequate levels of the amino acid lysine and therefore complement cereal based diets, which are typically deficient in lysine.

During the last decade, yields of dry beans have remained stationary. Some increases in production have occurred due to expansion of the areas under cultivation, but this cannot long continue, since new land suitable for agricultural development is limited. If this crop is to continue to play its important role in human nutrition, bean yields must increase in proportion to the rate of population growth.

Charles Darwin (1877) demonstrated that P. vulgaris is a self-pollinating species. He found that bean plants placed under insectproof netting produced as many seeds as plants visited by bees. These results have been confirmed more recently by Free (1966). Investigations of the extent of outcrossing in the common bean support the contention that it is highly self-pollinating. Observations of the amount of outcrossing in bean varieties range

from 0% to 13%, the higher value being unusual (Kristofferson, 1921; Mackie and Smith, 1935; Barrons, 1938; Tucker and Harding, 1975). It has been suggested that outcrossing occurs in P. vulgaris only because foreign pollen tubes grow more quickly than those of self-pollen (Free, 1970). Even if this is true, successful outcrossing is evidently a rare event in common beans under normal circumstances.

Because P. vulgaris reproduces by self-pollination, the species consists essentially of a large number of genetically pure lines. Variability within the species is great, but consists almost exclusively of differences between lines, which, for all intents and purposes, are reproductively isolated. In order to breed beans that yield greater harvests than current cultivars, it will be necessary to identify the best characters of many homozygous lines and to combine them into superior genotypes. Among the characters selected will be many that are quantitatively inherited, including adaptation to climatic variables, proper plant morphology, resistance to disease and pests, as well as high yield potential.

The traditional approach to combining a large number of characters in a self-pollinated crop can be illustrated with an example from the breeding of green beans. Unlike dry bean production, the bulk (65%) of green beans is produced in developed nations (FAO, 1978). Buyers of green beans for canning and processing adhere to a restrictive set of quality standards to which new cultivars must also conform. These include such pod characters as size, shape, color and fiber content, as well as seed color and agronomic requirements. Thus, a complex phenotype must be maintained in a breeding

program in addition to improving the type. Pedigree breeding, followed by mass selection or bulking, is inefficient if the materials used in the cross differ greatly in quality factors, and it is further complicated if the desired improvement is a quantitatively inherited trait. A very large, segregating population is required to recover the improved type, and consequently, a large number of manual cross-pollinations must be performed. The arduous and time-consuming task of emasculating and cross-pollinating beans by hand, on a large scale, makes such an approach impractical. Moreover, the single hybridization event and rapid approach to homozygosity in inbred generations limits recombination, reducing the probability of obtaining new, beneficial linkages.

Backcross breeding is an effective way to introduce simply inherited characters into established cultivars, and the end product closely resembles the recurrent parent genotype. However, if more than one improvement is contemplated, these must be introduced serially. Time and effort invested in this method are directly proportional to the number of simple traits to be added to the type.

In practice, most green bean breeding involves crossing elite lines in which quality factors are fixed, followed by selection for improved types. The genetic base in such breeding programs is necessarily narrow, and most present cultivars are considerably related. In 1970, 76% of all green-podded, bush bean seed produced in the United States was based on only three germplasm sources (Committee on Genetic Vulnerability, 1972). A narrow germplasm base creates an undesirable genetic uniformity for disease and pest resistance, rendering the crop vulnerable to epidemics.

The examples cited above point out a deficiency in breeding procedures for self-pollinating crops. An efficient method is needed to combine simultaneously many beneficial characters into single genotypes, utilizing a broad genetic base.

Population improvement procedures employing recurrent selection offer an alternative to traditional breeding procedures in filling this need. The initial step in a recurrent selection breeding program is the synthesis of a large, highly variable segregating population. A broad genetic base can be assured in the starting population by intercrossing diverse genotypes selected for heterogeneity in morphology, disease and pest resistance and agronomic merit. The purpose of the intercrossing step is to break existing linkages and promote recombination. In the segregating population that is produced, selection for beneficial linkages is made on the basis of maternal phenotype or, preferably, progeny tests. The selected plants furnish starting materials for the next cycle of intercrossing and selection. With each succeeding cycle, there is a concentration of useful genes in the population, and by intercrossing new linkage groups are formed. The cumulative result of this approach is a population in which beneficial genes, originally existing in different individuals, are concentrated and combined into single genotypes.

Because of the requirement for extensive intercrossing among many diverse genotypes, the above strategy has been applied mostly to the improvement of cross-pollinating crops. Population improvement procedures are equally applicable to self-pollinating crops, but as mentioned before, manual emasculation and cross-pollination

on a large scale make this approach hardly feasible. This is especially true for self-pollinating legume crops, since emasculation of papilionaceous flowers is delicate and tedious work, and the seed yield per pollination is low. It would be desirable to enhance the outcrossing ability of the common bean, in order to make full use of population improvement procedures in breeding programs. In this way, the burden of hybridization could be transfered primarily to bees, the natural agents of cross-pollination.

From the standpoint of utility in a recurrent selection breeding program, an ideal outcrossing mechanism should have several characteristics. Firstly, it should assure a high level of cross-pollination by insects, but also permit manual self-pollination if protected from insects. Thus, the genetic contribution of the male, as well as the female, can be controlled in materials selected for intercrossing.

Also, it should be simply inherited. Simple inheritance assures that a large proportion of the breeding population will segregate for outcrossing ability, thus facilitating selection for this trait. It also simplifies the removal of the outcrossing trait at the end of the improvement phase of the program, permitting the establishment of pure breeding lines.

The search for ways to promote outcrossing ability has already yielded some success in self-pollinating legume crops. Genic male sterility has been the most widely employed means toward this goal. Monogenic male sterility has been used in recurrent selection programs to facilitate intercrossing in cowpeas, Vigna unguiculata (L.) Walp. (Rachie et al., 1975; Rawal et al., 1978), and soybeans,

Glysine max (L.) Merr. (Brim and Young, 1971; Brim and Stuber, 1973). Similar male sterility has been identified in pigeon peas, Cajanus cajan (L.) Huth (Reddy et al., 1978), and its application in population improvement programs is being investigated. Rawal and Bassett (1976) have noted that at the end of a population improvement program, it is an easy matter to remove monogenic male sterility genes from any heterozygous line by selfing and selection, thereby creating self-pollinating pure lines. However, homozygous male sterile selections produce no viable pollen and are incapable of self-pollination, a disadvantage, as mentioned previously.

In common bean germ plasm, no effective, monogenic male sterility system has been described, although a report of a 2-gene system has recently been published and may prove useful in the future (Mutschler and Bliss, 1980).

A second kind of outcrossing mechanism is based on altered floral morphology. Structural changes isolating the stigma from the anthers, produce flowers that fail to shed pollen directly on the stigma. This kind of aberration has been described in cowpeas (Rachie et al., 1975). The anthers, which contain viable pollen, remain enclosed within abnormally constricted petals, while the style and stigma protrude. This character is simply inherited, but may have only limited application as an outcrossing mechanism, since the abnormal flowers are reported to be unattractive to pollinating insects.

Other reports of abnormal flower structures related to outcrossing ability have been published for peanuts, Arachis hypogaea L.

(Leuck and Hammons, 1969), and common beans (Rutger and Beckham, 1970). These floral anomalies have a genetic basis in that they occur more frequently in certain cultivars than in others. However, they are apparently much influenced by environmental factors, since normal, as well as abnormal, flowers bloom on the same plant. No analysis of inheritance has been attempted.

A suggestion that self-incompatibility factors may also be used to promote outcrossing (Denna, 1971) has so far found no application among self-pollinating legume crops.

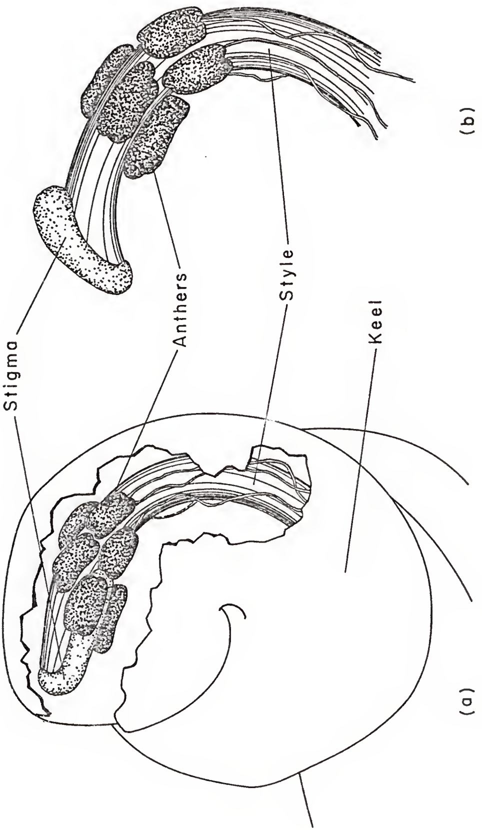
Since germ plasm within the species currently provides no means of improving outcrossing ability in P. vulgaris, it is necessary to examine other members of the genus for prospective sources of outcrossing genes. The scarlet runner bean, P. coccineus L., is perhaps the closest phylogenic relative of the common bean, as evidenced by the relative ease with which the two species hybridize. No genes for male sterility have been reported in P. coccineus, and while Lamprecht (1941) indicated that self-incompatibility systems exist within the species, subsequent investigations have not supported that claim (Cardwell, 1961; Hawkins and Evans, 1973). Nevertheless, the scarlet runner bean is one of the few legume crops that is substantially cross-pollinating. Estimates of the extent of outcrossing range from 30% to 70% (Miranda and Evans, 1973; Smartt, 1976).

The basis of the outcrossing mechanism of P. coccineus lies in the relationship between the stigma position and anther placement. This may best be understood in contrast to the self-pollinating structure of P. vulgaris flowers. In the latter species, pollination

usually occurs in the floral bud during the predawn hours preceding the opening of the blossom. The anthers surrounding the tip of the style dehisce adaxially, depositing sticky pollen onto the receptive stigma, as well as into the stylar hairs below (Webster et al., 1977) (Fig. 1(a)). Pollen tube growth is rapid, and fertilization occurs within 8 or 9 hours (Weinstein, 1926), leaving little opportunity for outcrossing. After the floral bud opens, the pistil and diadelphous stamens remain enclosed within the tubular coil of fused keel petals. Nectaries at the base of the pistil are functional, and the flowers are much visited by bumblebees and honeybees (Free, 1966). When a bee alights on the left wing petal, from which side the nectary is more accessible, a mechanical trip mechanism causes the tip of the style to protrude from the keel, bearing the stigma and adhering pollen to the left and into the path of the pollinator (Fig. 2). Even then the relationship between the bee and the stigma is less than ideal for cross-pollination. The stigma lies on the adaxial (internal) side of the coiled style, while the sterile abaxial (external) surface is presented to the pollinating insect.

In most points of floral development and morphology, P. coccineus and P. vulgaris are very similar. Pollen is shed in the bud prior to opening, just as in P. vulgaris, but self-pollination is prevented by the placement of the anthers in a ring around the style below the level of the stigma (Hawkins and Evans, 1973) (Fig. 1(b)). Instead of contacting the receptive stigma, pollen is deposited into the stylar hairs in an immobilized clump, leaving the tip of the style, upon which the stigma perches at an angle, free of pollen. The

Figure 1. The spacial relationship between anthers and stigma surfaces in flowers of *Phaseolus vulgaris* and *Phaseolus coccineus*. (a) In *P. vulgaris*, anthers lie in contact with the stigma surface, and self-pollination is effected at anthesis. (b) In *P. coccineus*, anthers shed pollen into stylar hairs (not shown) inferior to the level of the stigma, leaving the stigma surface unpollinated.


Stigma
Anthers
Style
Keel
(a)
(b)

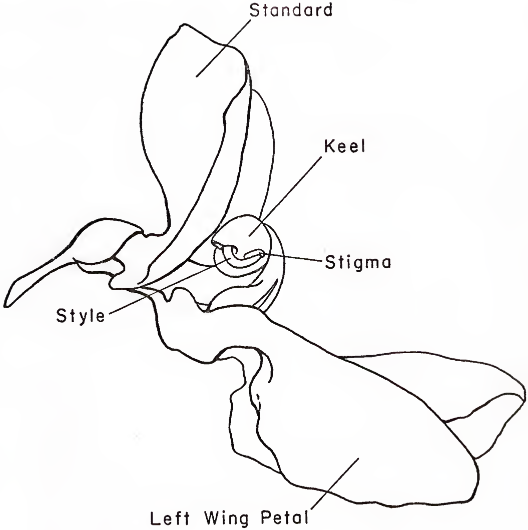


Figure 2. Spacial relationships of reproductive structures in the flower of the common bean, Phaseolus vulgaris. The left wing petal is depressed, as by the weight of a visiting insect pollinator, causing the extrusion of the style and stigma from within the protective keel petals.

stigma margins are bordered by a fringe of hairs, which further shields it from pollen contact in the bud (Ibrahim and Coyne, 1975). Upon opening, flowers of P. coccineus display a trip mechanism similar to that described in P. vulgaris, whereby foraging insects cause the tip of the style to protrude from the keel. However, whereas the stigma of P. vulgaris is introrse, lying on the internal side of the coiled style, in P. coccineus it is extrorse, being slanted across the tip of the style and down the external side (Lamprecht, 1941). The trip mechanism and the external stigma position combine to rub the stigma, which is initially free of pollen, squarely along the proboscises of bees attempting to feed from the nectary. In this manner, the stigma collects whatever foreign pollen the bees may be carrying (Farrer, 1868; Ogle, 1870). As an insect struggles to reach the nectary, the stigma sweeps past and upwards, followed by the brush of stylar hairs loaded with fresh self-pollen, which recoats the bee's body. When the pollinator leaves a flower, the style with pollen brush and stigma, retreats inside the keel once again. Upon repeated flexing of the wing petals, self-pollen is transferred to the stigma, so that in areas where insect activity is high, foreign pollen is probably in competition with self-pollen for unfertilized ovules (Darwin, 1857). This mechanism is well adapted to provide for a high level of outcrossing, since foreign pollen is normally deposited on P. coccineus stigmas at the same time as, or before, self-pollen.

The outcrossing flower structure of P. coccineus is a mechanism which might be incorporated profitably into P. vulgaris germ plasm

to enhance outcrossing in the common bean. This mechanism performs its function through the perfect orchestration of a multitude of subtleties, but for practical breeding purposes, it is necessary to identify only one, or a few, aspects of the structure, which will reproduce most nearly the desired behavior when introduced into *P. vulgaris* germ plasm. In the present work, the extrorse stigma has been selected as the single most important component of outcrossing structure. The external position provides the proper orientation of stigma and pollinator for effective cross-pollination. In addition, the extrorse stigma is shifted toward the tip of the style, where it is less likely to receive self-pollen in the bud. Consequently, the chief purpose of this study has been to transfer the gene, or genes, responsible for the extrorse stigma type from *P. coccineus* into *P. vulgaris* germ plasm. Also included in the breeding program have been experiments designed to provide information about the mode of inheritance of stigma position in interspecific materials.

The placement of anthers relative to the stigma is another structural variable of major importance in determining outcrossing ability. Anthers that dehisce at, or near, the tip of the style, as in *P. vulgaris*, assure a high level of self-pollination, while anther dehiscence inferior to the stigma favors outcrossing. However, anther placement is a difficult character to quantify, particularly in semisterile interspecific progenies, which shed only a limited amount of pollen. For this reason, no attempt was made to study anther placement.

LITERATURE REVIEW

Reciprocal Differences in Interspecific Crossings of *P. vulgaris* and *P. coccineus*

Superficially, *P. coccineus* closely resembles *P. vulgaris*. The major points of morphological divergence include flower color, stigma position and cotyledon position. *P. vulgaris* is characterized by white or lavender flowers, an introrse stigma and seedlings with epigeal cotyledons. *P. coccineus*, on the other hand, has scarlet or white flowers, an extrorse stigma and seedlings with hypogeal cotyledons. Other characters that differ are growth habit, the root system, the number of seeds per pod, and the length of the flowering raceme. *P. vulgaris* is an annual bush or vine with a fibrous root system, 6 to 9 seeds per pod and short racemes bearing 1 to 5 flowering nodes. *P. coccineus* is a vining perennial with tuberous roots, 4 to 6 seeds per pod, and long racemes bearing numerous flowering nodes. Seeds and pods of *P. coccineus* cultivars are larger than those of *P. vulgaris*.

In order to place the present study in perspective, it will be helpful to review the general behavior of interspecific crosses between these two species. The first report of a successful interspecific cross involving *P. vulgaris* and *P. coccineus* was that of Mendel (1866/1965) who produced the hybrid using *P. vulgaris* as the female parent. He noted that the F_1 plants bore a closer resemblance

to the pollen parent, P. coccineus, than to the maternal parent. The fertility of the hybrid plants was described as very slight. This cross has been repeated by numerous investigators with varying degrees of success, depending partly upon the parental lines employed. Some combinations yield no F_1 seed, while others produce 2 or 3 seeds per pollination (Lamprecht, 1941). With P. vulgaris as the female parent, embryo abortion is generally not greater than that encountered in intervarietal crosses, and plump F_1 seed with good germination can be obtained. The hybrid progeny, as Mendel observed, resemble P. coccineus in their vigorous vining habit, inflorescence structure and pod shape. However, many characteristics, including flower color, stigma position and cotyledon position, are intermediate between those of the parent species. Subvital genotypes, producing dwarf plants and seedling lethality, are reported in the F_1 generation (Lamprecht, 1941; Kedar and Bemis, 1960; Thomas, 1964; Smartt, 1970). These may appear in uniform progenies or in progenies segregating for subvitals. The latter condition results from heterozygosity in the interacting genes in the P. coccineus parent. Sterility in F_1 plants, as determined by pollen stainability, is in the range of 60% to 90% (Lamprecht, 1941; Smartt, 1970; Cheng, 1979). Consequently, manual pollination is required in order to produce seed. Seed production usually varies from 5 to 15 seeds per F_1 plant (Lamprecht, 1941). The F_2 generation contains many subvital and sterile individuals, the result of hybrid breakdown.

In contrast to the cross employing P. vulgaris as the female parent, the reciprocal cross is notoriously difficult to produce.

Many investigators have attempted the reciprocal cross without success, and the literature contains poorly researched references alluding to hybrids produced on P. coccineus, which in fact are inaccurate. Neither Tschermak (1933, 1942) nor Lamprecht (1966) was able to produce mature, flowering P. coccineus X P. vulgaris hybrids. Lamprecht did obtain two stunted plants, after making about 3600 cross-pollinations on P. coccineus, but these died as young plants. Al-Yasiri and Coyne (1966) considered both the P. vulgaris X P. coccineus cross and the reciprocal to be compatible. They obtained "mature pods" in 11% of the cross-pollinations made on P. coccineus. However, no information was given regarding quantity or viability of the F_1 seed, nor was any reference made to F_1 plants or their characteristics. Ibrahim and Coyne (1975) claim to have produced P. coccineus X P. vulgaris hybrids using White's solution on cross-pollinated stigmas of P. coccineus. However, the F_1 progenies, and at least one F_2 population, were uniformly identical to the female parent in all reported characters, suggesting self-pollen contamination. A few individuals in separate F_2 populations displayed white flowers or epigeal cotyledons, and these may represent authentic hybrids.

Although there are several earlier reports of successful reciprocal crosses, the earliest available to the present researcher is the work of Kroh (1962). In this investigation, P. coccineus X P. vulgaris plants were obtained with the aid of embryo culture techniques. Later, Thomas (1964) and Smartt (1970) produced the reciprocal cross without recourse to embryo culture. Smartt, Haq and

Nassar (1974) suggest that it may be very difficult to produce a P. coccineus line that is generally compatible, as a female parent, with P. vulgaris lines. Rather, individual genotype differences seem to be important. Certain combinations "nick" well, while the great majority do not.

Several hypotheses have been proposed to explain the difficulty in obtaining P. coccineus X P. vulgaris hybrids. Lamprecht (1941) examined the growth of P. vulgaris pollen tubes through P. coccineus stylar tissue, and could see no penetration beyond the superior half of the first stylar loop. He attributed the difficulty to a blockage mechanism in P. coccineus styles which prevents the passage of P. vulgaris pollen tubes. Thomas (1964) also suggested poor growth of pollen tubes of P. vulgaris in P. coccineus styles to explain lower hybrid pod set on P. coccineus than in the reciprocal cross on P. vulgaris. However, more recent investigation, using a combination of phase contrast and ultraviolet florescence microscopy, has shown that the P. coccineus style is not a barrier to P. vulgaris pollen tube growth (Hawkins and Evans, 1973). The P. Vulgaris pollen tubes are readily observed only in the superior 4 to 5 mm of the P. coccineus style, where callose formation is greatest, but they do penetrate the remainder of the style and enter the ovules in a manner indistinguishable from P. coccineus pollen tubes. Hawkins and Evans suggest that the low pod set observed by Thomas (1964) in interspecific crosses on P. coccineus may have been due to failure of gametic fusion, rather than blockage of pollen tube growth.

A second observation by Lamprecht (1948) introduced another hypothesis to explain the barrier operating in the reciprocal cross. Inbred materials, derived from P. vulgaris X P. coccineus hybridizations, were backcrossed reciprocally to P. coccineus. It was found that while the amount of viable seed set on P. coccineus was low (0.036 seed per pollination), about half of the pollinations actually resulted in pod set and the initiation of ovule development. Growth of pods and ovules generally halted prematurely, resulting in ovule abortion and empty seed. The reciprocal backcross, employing the inbreds as seed parents, succeeded more frequently (0.8 seed per pollination) and showed none of the ovule abortion noted when P. coccineus was used as seed parent. Lamprecht reasoned that since the nuclear genome of the F_1 embroyos was identical, regardless of the direction of the cross, the reciprocal difference in cross-compatibility could not have been the result of differences in chromosomal content. He concluded that the nourishment of the hybrid ovules developing on P. coccineus had been disrupted for an unknown, extra-genic reason. These observations have been verified in reciprocal interspecific crosses by Thomas (1964).

The cause of ovule abortion in P. coccineus X P. vulgaris crosses was investigated by Kroh (1962) and Thomas (1964). These researchers made histological studies of hybrid ovule development on P. coccineus. They concluded that hybrid embryo development was retarded due to the inability of the hybrid endosperm tissue to provide a proper nutritive medium.

Contrary to Lamprecht's conclusion, reciprocal differences in physiological compatibility of hybrid embryo and endosperm tissues may have a genic basis (Smartt and Haq, 1972a). It is true that the diploid nuclei of the hybrid embryos have the same chromosome complement, regardless of the direction of crossing, but the triploid hybrid endosperm nuclei differ reciprocally in the ratio of their parental genomes. Alternately, cytoplasmic differentiation in the two species could result in lethal interaction with hybrid nuclei in *P. coccineus*, while presenting no barrier to embryo development in *P. vulgaris* (Smartt and Haq, 1972a). A third proposal attempting to explain reciprocal differences in ovule abortion hypothesizes the existence of inhibiting substances in the foliage of *P. coccineus*, which cause late embryo abortion when translocated to the developing pods (Ibrahim and Coyne, 1975).

Whatever the reason, a reciprocal difference in the success of hybrid ovule development does exist, and it corresponds to the reciprocal difference in interspecific cross-compatibility between the two species. Ovule abortion, coupled perhaps with a reduction in gamete fusion, seems to be the principal barrier to the production of F_1 hybrids on *P. coccineus*.

F_1 hybrids produced on *P. coccineus*, with or without the aid of embryo culture, resemble hybrids produced by the reciprocal cross in most respects. Morphologically, all have indeterminate vining growth, long flowering racemes and intermediate stigma and cotyledon positions (Thomas, 1964; Smartt, 1970). An exception is the F_1 material described by Ibrahim and Coyne (1975), which is perhaps best explained

as the product of self-pollen contamination, as mentioned previously. In addition to normal hybrids, the same types of subvital individuals, including dwarf necrotic plants and lethal seedlings, can be found in progenies resulting from crosses in either direction. These effects are attributed to allelic and genic interactions in the nuclear chromosomes (Kroh, 1962; Thomas, 1964; Smartt, 1970).

A purported difference between F_1 progenies produced reciprocally is the higher fertility attributed to hybrids having *P. coccineus* cytoplasm. Several reports have indicated that pollen stainability of *P. coccineus* X *P. vulgaris* F_1 plants ranges from 77% to 87%, as compared with 10% to 30% generally observed in the reciprocal cross (Smartt, 1970; Smartt and Haq, 1972*a*; Smartt et al., 1974). The increased pollen stainability is reflected in greater seed set and nearly normal Mendelian segregation ratios in the F_2 generation. The reciprocal difference in F_1 fertility has been ascribed to differences in parental cytoplasm or differences between hybrid endosperm tissue, or both. There are relatively few observations of fertility in *P. coccineus* X *P. vulgaris* hybrids, and conclusions based upon the data currently available may be premature. Thomas (1964) reported pollen stainability of 17% to 35% in progenies of reciprocal crosses, with no significant differences between the reciprocal F_1 progenies.

Hybrid breakdown in the F_2 generation is reported to be less severe in *P. coccineus* X *P. vulgaris* crosses than in the reciprocal (Smartt et al., 1974).

To recapitulate, *P. vulgaris* X *P. coccineus* crosses are characterized by nearly normal fertilization and hybrid seed development,

low F_1 fertility and marked F_2 hybrid breakdown. The reciprocal cross shows an apparent reduction in gamete fusion, a potent ovule abortion barrier, F_1 fertility approaching normal, and some F_2 hybrid breakdown. In the former cross, the major barriers to interspecific hybridization are expressed in the F_1 and later generations, whereas in the latter cross, the primary barrier operates in the maternal parent. This situation is close to unilateral incompatibility.

How this relationship of unilateral incompatibility has evolved is a question that has prompted interesting speculation. Primitive, wild forms of _P. vulgaris_ and _P. coccineus_ in Mexico and Central America are effectively isolated from each other by virtue of their adaptation to different environments. Wild _P. vulgaris_ is most commonly found at medium elevations, while wild _P. coccineus_ is native to higher elevations and cooler, moister conditions (Freytag, 1979). That geographic isolation is the chief barrier to interspecific hybridization in wild populations is indicated by the work of Miranda and Evans (1973), in which wild plants of _P. vulgaris_ and _P. coccineus_ were found to cross freely and with equal ease in either direction. The development of unilateral incompatibility which characterizes the crossing behavior of domesticated _P. vulgaris_ and _P. coccineus_ has resulted from two factors (Smartt and Haq, 1972_a_). The first of these was the influence of man, the cultivator, in bringing the two species together in close physical proximity. Smartt and Haq note that common beans and scarlet runner beans are frequently grown in adjoining fields in northwestern Europe and the British Isles today. Surely, this must also have been true in Mexico and Central America

for the past several millenia. Smartt and Haq suggest that the response of *P. coccineus* to the loss of geographic isolation differed from that of *P. vulgaris* as a result of a second influence--differing reproductive strategies in the two species. Because of the importance of outcrossing in the reproduction of *P. coccineus*, its gene pool is vulnerable to disruption from unrestricted hybridization with related compatible species. In situations where a large portion of a generation may be lost in the production of inviable or sterile hybrids, strong selection pressures for effective genetic isolating barriers are operative. In *P. vulgaris*, on the other hand, outcrossing is a relatively rare occurrence. The bulk of seed produced on any individual preserves the integrity of the parental genotype. Genetic isolating mechanisms provide no reproductive advantage in the case of *P. vulgaris*, since it is already isolated through the mechanism of its floral morphology. These factors have led to the development of unilateral incompatibility in which genetic barriers to hybridization are present in *P. coccineus* cultivars, but are lacking in *P. vulgaris* cultivars.

Other work indicates that evolution in *P. vulgaris*, as well as in *P. coccineus*, has contributed to the development of reciprocal differences in cross-compatibility. In crosses of a temperate region cultivar of *P. coccineus* with both primitive and domesticated *P. vulgaris*, the usual reciprocal differences in cross-compatibility were evident as expected (Miranda and Evans, 1973). However, in crosses of a primitive *P. coccineus* line with both wild and domesticated *P. vulgaris*, only the cross between primitive lines was free

of reciprocal differences in cross-compatibility. If evolutionary changes in the P. coccineus genome alone had been responsible for the development of incompatibility reactions when P. coccineus serves as the seed parent, the crosses involving primitive P. coccineus should have been free of reciprocal compatibility differences, regardless of the P. vulgaris germplasm source.

F_1 Sterility, Selective Elimination of Genotypes, and Distorted Segregation Ratios in P. vulgaris X P. coccineus Hybrids

Because of the great difference in cross-compatibility in reciprocal crosses, the bulk of research dealing with hybridization between P. vulgaris and P. coccineus has involved crosses using P. vulgaris as the maternal parent. Characteristics of the F_1 generation have been mentioned previously. Among them, the reduced F_1 fertility deserves further elaboration, with respect to its cause and its effect on segregation in F_2 and backcross generations.

The sterility of F_1 hybrids produced in P. vulgaris X P. coccineus crosses has been noted repeatedly in the literature. Generally, no seed is set on F_1 plants without manual pollination. Even with artificial pollination, seed set is low, indicating that the observed sterility is due to factors other than structural mechanisms restricting self-pollination, such as might be expected in hybrids of outcrossing P. coccineus and self-pollinating P. vulgaris. Typical pollen stainabilities of 10% to 30% confirm that many abnormal and inviable gametes result from meiosis in F_1 plants.

The earliest studies of meiotic behavior in P. vulgaris X P. coccineus F_1 hybrids were made by Karpechenko (1925) and Lamprecht (1941). Apart from occasional groups of 2 or 3 bivalents at metaphase I, which Lamprecht mentioned as possible secondary associations, these investigators found that the hybrids followed a normal meiotic sequence with no indication of univalent formation, structural differentiation or disturbances. Nevertheless, the majority of pollen tetrads was observed to degenerate after meiosis (Lamprecht, 1941). These results formed the departure point for subsequent investigations, which attempted to explain the F_1 sterility in light of the apparent lack of important structural differences in the genomes of the two species.

The publications of Lamprecht in the 1940's offered the first hypothesis treating the cause of F_1 sterility and its effect on segregation in F_2 and higher generations. He noted that, due to recombination, gametes produced by P. vulgaris X P. coccineus F_1 hybrids vary in their genomic constitution from types similar to P. vulgaris to types resembling P. coccineus. Lamprecht (1941) suggested that the gametes most likely to survive and produce progeny would be those having genomes similar to the P. vulgaris maternal parent, since these would function most harmoniously in the gametic cytoplasm of maternal origin. This hypothesis accounted for the sterility observed in F_1 plants having apparently normal meiotic behavior, as well as for the elimination of P. coccineus characters in the F_2 generation.

Lamprecht (1944) later modified this concept to account for his observation that, in reality, most P. coccineus genes can be transferred successfully into P. vulgaris cytoplasm; and inbred lines can be isolated in advanced generations that represent recombinations of most morphological characters separating the two species. The genes controlling such characters he called intraspecific genes. On the other hand, there were two P. coccineus traits, namely the extrorse stigma and hypogeal cotyledon position, that he was never able to recover in fertile interspecific hybrids. The genes governing these characters he called interspecific genes. He hypothesized that interspecific genes were unable to replicate in alien cytoplasm. To support this hypothesis, he devised a conjectural mechanism of gene replication, which explained how interspecific genes could replicate in F_1 somatic divisions, but fail in gametic divisions following meiosis, thereby resulting in gene deletions, gamete inviability and the observed F_1 sterility. Lamprecht (1948) also made crosses onto P. coccineus, using as the pollen parent certain inbred lines derived from interspecific hybrids. Here he claimed the reciprocal effect, namely, that sterility in the F_1 with P. coccineus cytoplasm resulted in elimination of P. vulgaris interspecific alleles in the F_2 generation. However, no information was given regarding the stigma type or cotyledon position of the interspecific pollen parent used in these crosses.

While the observations of Lamprecht have generally been supported by more recent investigators, his interpretation of his work, particularly the distinction between intraspecific and interspecific

genes and the now outmoded hypothesis of gene replication, has been received with skepticism. A common theme of writers commenting upon Lamprecht's work has been the contention that interspecific barriers separating the two species are not pleiotropic effects of genes controlling stigma and cotyledon position. Stebbins (1950) was the first to suggest that the factors controlling morphological differences between the species were separate from those responsible for interspecific sterility. He proposed that linkage was the cause of the apparent intimate association and predicted that in large interspecific progenies, the linkage might be broken to give fertile plants with the missing phenotypes.

As an example of this kind of linkage, Stebbins referred to the work of Hiorth (1942) and Hakansson (1947) involving interspecific crosses between Godetia amoena and G. whitneyi. These species have identical haploid chromosome numbers (n=7), and in interspecific hybrids, six bivalents and one pair of univalents were usually observed at meiotic metaphase I. The hybrids were highly, but not completely, sterile. By backcrossing to the parental species, Hiorth found that most morphological characters were transferable from one species to the other. An exception was a species-distinguishing character, petal spot position, which could not initially be transferred without accompanying sterility. However, backcrossing to G. whitneyi eventually produced fertile plants that displayed the petal spot position of G. amoena. The allele responsible for this trait had been transferred by crossing over to the homeologous G. whitneyi chromosome without the sterility-inducing genes originally linked to it.

Grant (1967) proposed a similar explanation of Lamprecht's work, involving linkage of morphological genes with genes affecting viability of the male gametophyte. He suggested that P. vulgaris and P. coccineus carry different members of a set of complementary factors which interact to bring about pollen lethality, with the pollen lethal factors of P. coccineus being linked with stigma and cotyledon alleles of that species.

Experimental evidence from several sources indicates that hybrid inviability and sterility are not pleiotropic effects of stigma and cotyledon position genes. In a compex interspecific breeding program involving P. vulgaris, P. coccineus, and P. lunatus, Honma and Heeckt (1963) claimed success in transferring the hypogeal cotyledon trait of P. coccineus into fertile hybrid segregants on P. vulgaris cytoplasm. The authors suggested that genes controlling cotyledon position in these species operate independently of the cytoplasm, contrary to Lamprecht's notion.

Smartt agreed that the arguments of Stebbins and Grant were more convincing than Lamprecht's in explaining the relationship between the interspecific barrier and genes controlling stigma and cotyledon position. He stated that these loci "do not necessarily control species differentiation, but may mark chromosome segments which do affect hybrid viability and/or fertility" (Smartt, 1970, p. 487). In addition, he noted that strong selective elimination operated similarly against the indeterminate growth habit of P. coccineus in the interspecific F_2 generation.

Le Marchand (1971) has produced interspecific hybrids involving P. vulgaris, P. coccineus, P. polyanthus Greenm., P. obvallatus Schlecht., and P. formosus Knuth. The latter four taxa are closely related and recently have been described as subspecies of P. coccineus (Marechal et al., 1978). Le Marchand reported that interspecific hybrids between P. vulgaris and members of the P. coccineus complex, other than P. coccineus itself, showed no lethality or compatibility barrier, but rather a marked heterosis and relatively good fertility. He pointed out that if the genes governing stigma and cotyledon position are responsible for the compatibility barrier in P. vulgaris X P. coccineus crosses, as Lamprecht had concluded, then these loci can lose their lethal character in crosses to other members of the complex, which also have hypogeal germination and stigmas more extrorse than those of P. vulgaris.

Finally, a brief note by Bannerot (1979) indicated that the author had successfully transferred the nuclear genome of P. coccineus into P. vulgaris cytoplasm, presumably through a backcrossing program using P. coccineus as the recurrent male parent. Since the resulting population was reported to be partially allogamous, yielding spontaneous hybrids under field conditions, individuals must have possessed relatively high fertility, as well as the outcrossing stigma type of P. coccineus. The existence of this combination on P. vulgaris cytoplasm contradicts Lamprecht's hypothesis and casts doubt upon a direct relationship between genes controlling stigma position and hybrid sterility and inviability.

Interspecific crosses between the cultivated amphidiploid Gossypium species, G. barbadense L. and G. hirsutum L., share some similarities with hybrids involving P. vulgaris and P. coccineus; and in a general sense, the interspecific barriers operating in the two systems may be comparable. The Gossypium hybrids have no observable meiotic abnormalities, and chromosome pairing is regular. While F_1 plants are vigorous and fully fertile, the F_2 generation shows marked hybrid breakdown. Depressed vigor, weak seedlings, and sterile and physiologically unbalanced individuals are common in F_2 populations. Only segregants resembling the parental species become stabilized under human or natural selection. Intermediate types, resembling the F_1, again produce unbalanced progeny in the F_3 generation. This behavior was observed also in hybrids between the diploid cultivated species G. arboreum L. and G. herbaceum L.

On the basis of observations involving the Gossypium species, Harland (1936) proposed that differences between species are primarily the result of differences in "genetic architecture." New species arise through the action of natural selection, constructing integrated systems of modifier complexes which are unique to each species. Interspecific hybridization results in modifier segregation in F_2 and higher generations, with a consequent mutual disruption of the two internally balanced parental systems. As a result, inbred progenies consist of inferior, unproductive segregants.

Hutchinson (1971) noted the similarity of the genetic relationship between P. vulgaris and P. coccineus, on the one hand, and between the Gossypium species, on the other. He commented that

Harland's hypothesis does seem to apply to the Phaseolus species as well, but mentioned that reciprocal differences in Phaseolus crosses indicate an additional cytoplasmic influence not found in Gossypium crosses.

The separate influences of genic unbalance and genic-cytoplasmic interaction in Phaseolus hybrids have been investigated in experiments designed to probe the nature of interspecific barriers. Smartt (1970) concluded that a mechanism similar to that proposed by Harland is partly responsible for sterility in P. vulgaris X P. coccineus F_1 hybrids. Sterility in the F_1 generation involves abortion of pollen grains, and perhaps also embryo sacs, having inviable genotypes. Inviability arises from loss of genetic balance as a result of crossing over between parental chromosomes and random assortment of whole chromosomes.

Smartt explained the shortage of F_2 segregants resembling P. coccineus by the statement that "P. vulgaris genes above a certain frequency apparently reduce the viability of P. coccineus genomes in a P. vulgaris plasmon" (Smartt, 1970, p. 487). Rare F_2 gametes containing predominatly P. coccineus genes will form viable zygotes in P. vulgaris cytoplasm only when they fuse with gametes of similar, infrequent genetic constitution.

In addition, Smartt resurrected and modified Lamprecht's notion of interaction between alleles of P. coccineus and cytoplasm of P. vulgaris. Fertility in F_2 progeny was reported to be under cytoplasmic control, with segregants resembling P. vulgaris having higher fertility than those resembling P. coccineus. Smartt suggested that

genome and cytoplasm must have a common origin to ensure full fertility. In this statement, Smartt echoes the earlier work of Wall and York (1957). These investigators noticed a persistent shift toward the epigeal cotyledon position of P. vulgaris in F_2, F_3 and F_4 interspecific hybrid populations. They attributed this effect to a gradual increase in the frequency of P. vulgaris alleles in succeeding inbred generations, brought about by greater fertility of genotypes resembling P. vulgaris. A selective advantage of gametes, and/or zygotes, with a high proportion of P. vulgaris alleles in P. vulgaris cytoplasm was offered as a possible reason for the elimination of P. coccineus types. As Hutchinson (1971) noted, the apparent differentiation in cytoplasm, as well as in the nucleus, may represent a step beyond Gossypium in the evolution of interspecific barriers toward complete incompatibility.

According to Harland's hypothesis, speciation depends primarily upon shifts in frequencies of modifier alleles, without effect upon the basic organization of the loci involved. Thus, there is no reason why the chromosomal structure of daughter species, differentiated by this mechanism, should not correspond exactly, locus for locus. Yet certain genetic, cytological and breeding phenomena in interspecific Gossypium hybrids are more readily explained on the basis of small differences in parental chromosome structure than in terms of genic unbalance. Stephens (1950) has reviewed the information pertinent to the conflict of theory and has proposed an alternate interpretation of interspecific barriers that is based on the existence of cryptic structural differentiation in the genomes of closely related Gossypium species. The phrase "cryptic structural

differentiation" refers to small scale transpositions, translocations and inversions that involve such minute chromosome segments that they do not produce abnormal meiotic configurations, such as multivalents and bridge fragments, in hybrids. However, crossing over between structurally differentiated chromatids in F_1 hybrids does produce minute duplications and deletions, with the result that gametes and zygotes carrying a high proportion of crossover chromatids will generally be less fit than those bearing predominatly non-crossover chromatids. This is the mechanism proposed by Stephens (1950) to explain the occurrence of F_2 breakdown and recovery of parental types through stabilizing selection in Gossypium hybrids. Among the evidence presented as indicative of cryptic structural differentiation in these hybrids is preferential genomic pairing in synthetic allotetraploids, selective elimination of donor parent genes in certain interspecific backcrosses, and the extraordinary rapidity with which the phenotype of the recurrent parent is recovered in backcrosses.

Because the genetic relationship between P. vulgaris and P. coccineus has been compared with that between Gossypium species, as mentioned previously, the possible significance of cryptic structural differentiation in Phaseolus hybrids is a matter worthy of comment. The apparently normal meiotic behavior of P. vulgaris X P. coccineus F_1 hybrids (Karpechenko, 1925; Lamprecht, 1941) does not conclusively rule out the possiblility that numerous small regions of structural heterozygosity may exist in the hybrid genome. Stebbins et al. (1946) have concluded from their studies that two chromosomes may differ by a maximum of 5 or 6 segments large enough to produce lethality or

semilethality when deleted from gametic genomes, and yet not betray evidence of structural hybridity through irregular pairing or reduced chiasmata formation. Since the P. vulgaris X P. coccineus hybrid has 11 pairs of chromosomes, as many as 55 to 66 translocated or inverted segments could perhaps exist in the genome without obvious disruption of meiosis. Structural heterozygosity for only 4 or 5 such segments could be sufficient to produce a high degree of hybrid sterility (Sax, 1933). Thus, cryptic structural hybridity, as well as genic unbalance, must be considered capable of explaining the phenomenon of sterility in F_1 hybrids with normal meiotic sequences.

There are several pieces of information in the literature that seem to indicate that the genomes of P. vulgaris and P. coccineus may differ structurally, as well as genically. Wall (1968) has shown that segregation for species-specific, polymorphic forms of leucine aminopeptidase in P. vulgaris X P. coccineus hybrids is controlled by codominant alleles at a single locus. Reciprocal backcrosses to the parent species revealed that selective elimination of the donor parent allele occurs through F_1 male gametes, but not through female gametes. The same phenomenon occurs in reciprocal backcrosses to P. vulgaris for alleles governing cotyledon position, a trait shown to be quantitatively inherited (Wall and York, 1957; Wall, 1970). Wall (1968) indicated that pollen competition is probably responsible for a generalized exclusion of the donor parent genome in interspecific Phaseolus backcrosses. This contention was supported with evidence that backcross progeny derived from F_1 male gametes are more fertile, and therefore have more compatible genotypes

(i.e., genotypes more nearly like the recurrent parent), than backcross progeny obtained from F_1 female gametes.

This sort of behavior is very similar to the situation described in Gossypium hybrids, with the exception that the female gametes in the latter are also frequently involved in selective elimination of genes (Stephens, 1949). Stephens' (1949) explanation of selective elimination of the donor genome, to which Wall (1968, 1970) also subscribed, is based upon the hypothesized existence of small, differentiated chromosome segments in the F_1 interspecific hybrids. Viable and competitive gametes from such plants tend to contain mostly non-crossover chromatids, since crossing over results in deleterious duplications and deletions in regions of structural heterozygosity. In backcrosses to parental species, pollen with genomes most nearly like that of the recurrent parent will compete most successfully in zygote formation, to the partial exclusion of pollen with predominatly donor parent chromatids. The latter statement was supported by evidence that cotton plants, when pollinated with a mixture of pollen from the same species and a closely related, cross-compatible species, consistently yielded a majority of nonhybrid progeny (Kearney and Harrison, 1932). Thus, it is suspected that selection of the P. vulgaris genome occurs in backcrosses of F_1 hybrids to P. vulgaris, and for P. coccineus genomes in backcrosses to P. coccineus. In Gossypium species, the active role of female gametes in selective elimination of donor parent genes was attributed to larger structural differences in the tested chromosome regions than in the Phaseolus hybrids (Wall, 1968).

It has been pointed out that skewed backcross ratios can be explained on other grounds, and do not, by themselves, indicate the presence of structurally differentiated genomes (Stephens, 1949). Systems of linked, internally balanced modifiers in donor parent chromosomes could suffer selective disadvantage in backcrosses, resulting in the same kind of elimination as described above. However, it is the rate at which the donor parent phenotype, as a whole, is eliminated in backcrosses, that suggests that modifier complexes are both linked and structurally differentiated. In cotton, progenies closely resemble the recurrent parent after only two or three backcrosses (Knight, 1945). Structural differentiation reduces recombination of parental genomes, and those crossover genotypes that do occur are eliminated because of lethal and subvital duplications and deletions. This makes selection for parental genomes more efficient and results in rapid elimination of the donor influence.

Information is not available concerning the rate of loss of the donor parent phenotype in _Phaseolus_ interspecific backcrosses. However, Wall (1970) has traced the elimination of _P. coccineus_ alleles for hypogeal cotyledon through three consecutive backcrosses, using _P. vulgaris_ as a recurrent female parent. Even though selection intensities of 10% to 20% were employed for the hypogeal trait in each backcross, the distribution of cotyledon positions after only two backcross generations closely resembled the distribution of the epigeal _P. vulgaris_ parent.

Tetraploid interspecific hybrids have been created by treating _P. vulgaris_ X _P. coccineus_ F_1 plants with colchicine. In higher

generations, segregation for flower color, seed coat color, and fertility was observed, indicating that these plants behave as segmental allotetraploids (Wall, 1970; Smartt and Haq, 1972_b_). After an initial jump in fertility from an average of 21% in the interspecific F_1 to an average of 42% in the raw amphidiploid, inbreeding and segregation produced a broad range of fertilities from 3% to 64% in the C_3 generation. The less fertile plants were lost, and by the C_5 generation, fertility as high as 76% had been achieved. Smartt and Haq (1972_b_) attributed control of fertility in the hybrids to both chromosomal and genic influences. The initial increase in fertility following colchicine treatment was explained as the result of preferential pairing of homologues. Homoeologous pairing was also said to occur with subsequent recombination and segregation for genes affecting fertility. This resulted in upward and downward variation from the mean fertility of the raw amphidiploid.

Stebbins (1958) pointed out that it is very difficult to determine whether the sterility in interspecific hybrids stems from genic or chromosomal causes. An increase in average fertility upon chromosome doubling is not necessarily an indication that structural hybridity exists in the parental genomes. Such an increase would also be expected if sterility resulted from a system of complementary gene pairs, since the proportion of fertile genotypes is larger in tetrasomic segregation ratios than in diploid segregation ratios. The conclusion of Smartt and Haq (1972_b_), that the chromosomes of _P. vulgaris_ and _P. coccineus_ are structurally differentiated, must be considered speculative.

Contrary to the early reports of Karpechenko (1925) and Lamprecht (1941), there is now cytological evidence indicating that meiosis in P. vulgaris X P. coccineus F_1 hybrids does not follow a completely normal course. One pair of univalents is occasionally seen at metaphase I in the parental species, as well as in the hybrid, and a maximum of three pairs of univalents has been reported in F_1 hybrids (Marechal, 1971; Cheng, 1979). Also, the average number of chiasmata per bivalent is somewhat reduced, compared with the parental species. However, these anomalies are attributed to genic interactions or cytoplasmic influences, rather than structural heterozygosity (Marechal, 1971).

At anaphase I and metaphase II, occasional cells are noted with 10 chromosomes at one pole and 12 at the other. The unequal distribution may be due to asynapsis, followed by the movement of both univalents to a common pole, but it is thought more likely that nondisjunction is involved (Cheng, 1979).

Most interesting of all the recent cytological findings has been the observation of chromosome bridges at anaphase I and anaphase II in P. vulgaris X P. coccineus hybrids. According to Cheng (1979), at least two, large paracentric inversions exist in the F_1 materials studied by him, and these accounted for 20% to 40% of the observed F_1 pollen abortion. The remainder was attributed to genic unbalance in gametes.

The transmission of any chracter from P. coccineus into P. vulgaris germ plasm may be opposed by barriers of sterility,

inviability and selective elimination. The same barriers prevent precise determinations of the mode of inheritance of characters in interspecific hybrids, since genetic analysis is hindered by insufficient progeny sizes and distorted segregation ratios. According to Wall (1968), the functional basis of these barriers probably involves both cryptic structural differentiation and genic unbalance. To these, now also may be added genic-cytoplasmic interactions and large paracentric inversions.

Inheritance of Stigma Type in Interspecifc Crossings of *P. vulgaris* and *P. coccineus*

The nature and degree of fertility in *P. vulgaris* X *P. coccineus* F_2 and F_3 progenies was examined over half a century ago by comparing seed set on plants exposed to insect pollinators with seed set on protected plans (Ten Doornkaat Koolman, in Kooiman, 1931). Fertility was classified in three categories:

1) Plants rather fertile when protected, as well as when exposed to insects.

2) Plants infertile when protected, as well as when exposed to insects.

3) Plants infertile when protected, but fertile when exposed to pollinators.

These types occurred in a ratio of 9 : 8 : 14, respectively. The third, and most abundant, category seems to have possessed some degree of outcrossing ability. There was no mention of the relationship of stigma position to the fertility categories, except

for a statement that both "P. vulgaris" and "P. coccineus" flower types could be either self-fertile or self-sterile.

Kooiman (1931) first suggested a study of stigma position in relation to anther position in interspecific generation segregating for sterility. However, it was Lamprecht (1941) who first attempted a genetic interpretation of the variation in stigma position in interspecific hybrids. He illustrated and described five stigma types, including the two parental extremes, an intermediate "pilleus" type which was characteristic of the interspecific F_1, and two forms falling between the "pilleus" stigma and the parental types. The F_2 population contained all five stigma types and other "difficult-to-classify intermediates" (Lamprecht, 1941, p. 116). Frequency distributions of stigma types in early generation, inbred progenies were skewed toward the introrse stigma type of P. vulgaris. In higher inbred generations, all stigma types bred true, with the exception of the F_1 "pilleus" form, which was considered to be heterozygous. When crossed with one another, or in backcrosses to either parent species, the homozygous, higher generation, inbred lines always produced F_1 progeny with stigma forms that were uniformly intermediate between the parental lines.

Lamprecht (1941) initially proposed a two-locus system to account for stigma inheritance. His system was essentially additive, since, in his own words, "what manifestation is assigned to dominant and recessive forms of either gene seems unimportant here, inasmuch as the heterozygote types are typically intermediate"

(Lamprecht, 1941, p. 117). As Lamprecht continued to develop his genic-plasmic concept of interspecific barriers, he modified the genetic model of stigma position inheritance to include one interspecific gene and one intraspecific gene, and finally reduced the scheme to a single interspecific gene, with only three phenotypic categories (Lamprecht, 1944, 1945, 1948, 1964). Self-pollination of the semisterile individuals classified as having the P. coccineus stigma type under the monogenic model of inheritance, always produced progenies with a majority of intermediate stigma types and a few P. vulgaris stigma types, as well as the expected P. coccineus stigma types. Lamprecht (1944) interpreted these results to indicate that the interspecific gene for the P. coccineus stigma form had mutated at a high rate to the corresponding P. vulgaris stigma gene in hybrid cells with cytoplasm derived from P. vulgaris.

Another attempt to provide a genetic framework for stigma inheritance in Phaseolus hybrids was made by Miranda (1965). Using plant materials that had previously been described as products of natural hybridization between P. vulgaris and P. coccineus (Hernandez et al., 1959), he produced backcrosses to both parental species and inbred these to examine stigma segregation in the F_2 generation. Four phenotypic classes were described, including the two parental types, an "apical" stigma type found only in the backcrosses to P. coccineus, and a "semilateral" stigma type, intermediate between the apical and P. vulgaris types, which was characteristic of the natural hybrid. The backcross to P. coccineus

segregated in the F_2 generation in a ratio of 9 apical types ($S\_M\_$) : 3 P. coccineus types (S_mm) : 3 semilateral types ($ssM\_$) : 1 P. vulgaris type (ssmm), indicating that two independent loci with complete dominance were involved. The backcross to P. vulgaris (ssmm) indicated a single gene difference, with the semilateral stigma form (ssMM) being dominant. According to this model, the artificially produced P. vulgaris (ssmm) X P. coccineus (SSmm) F_1 hybrid ought to yield only extrorse stigmas (Ssmm) resembling P. coccineus. The fact that Miranda's natural hybrid had semilateral stigmas (ssMM) was explained in this work with the aid of two assumptions. Firstly, a dominant mutation occurred at a second locus and became fixed in the population sometime after the original hybridization event. Secondly, apical stigma types (S_MM) were lost in segregating populations due to associated lethal genes or genes producing reproductive isolation.

This model predicts F_1 and F_2 segregation ratios for artificial P. vulgaris X P. coccineus hybrids that are very different from those actually documented by Lamprecht (1941). The artificial hybrids, by which the accuracy of the model could have been checked, were produced by Miranda, but no data from these crosses were given, since, according to the author, they were still "in the process of evaluation" (Miranda, 1965, p. 195).

Smartt (1970) produced F_1 populations from reciprocal crosses of P. vulgaris and P. coccineus. The stigmas in all F_1 populations were intermediate between the parental species. Large F_2 progenies,

ranging from 53 to 150 individuals each, were produced. Segregating stigma types were classified in six phenotypic categories, ranging from the introrse <u>P. vulgaris</u> type to the extrorse <u>P. coccineus</u> type. Smartt stated that the size of his F_2 populations was insufficient for a proper genetic analysis; but contrary to Lamprecht's model, he indicated that stigma position is not a monogenically inherited trait.

As mentioned previously, Le Marchand (1971) has produced interspecific hybrids between <u>P. vulgaris</u> and members of the <u>P. coccineus</u> complex, taxonomically defined by Marechal et al. (1978). Stigma positions in these species and F_1 hybrids displayed a progressive, linear transition from <u>P. vulgaris</u>, the most introrse, to <u>P. obvallatus</u>, the most extrorse. According to Le Marchand, this suggested quantitative, rather than monogenic, control of this trait. Ibrahim and Coyne (1975) agreed, saying that inheritance of stigma position in <u>P. vulgaris</u> X <u>P. coccineus</u> hybrids studied by them was quantitative. The F_1 generation again showed an intermediate stigma position, and stigmas in the backcross to <u>P. vulgaris</u> were continuously distributed in the range between F_1 type and the introrse <u>P. vulgaris</u> type. However, in the reciprocal interspecific cross, a discontinuous pattern of inheritance was reported. The authors attributed this to cytoplasmic influences which affect the expression of stigma position genes differently in reciprocal crosses. An alternate explanation, mentioned before, is that the "reciprocal" progeny were not hybrids at all, but

resulted from contamination of *P. coccineus* female parent plants with self-pollen.

In summary, the stigma position of *P. vulgaris* X *P. coccineus* F_1 hybrids is reported to be intermediate between the parental stigma types. Segregation in the F_2 generation produces a range of stigma types and "intermediates," most of which breed true in advanced generations. The F_2 distribution is skewed toward the introrse stigma of *P. vulgaris*. The various genetic models that have been proposed to explain stigma inheritance include a two gene model with additive effects (Lamprecht, 1941), a single interspecific gene model (Lamprecht, 1945), a two gene model with complete dominance at both loci (Miranda, 1965), and several unelaborated suggestions of a quantitative mode of inheritance (Smartt, 1970; Le Marchand, 1971; Ibrahim and Coyne, 1975).

It should be noted that all of the previous work dealing with the inheritance of stigma position in *P. vulgaris* X *P. coccineus* hybrids has been based on qualitative assessments of stigma types. In typical studies, a more or less arbitrary set of idealized phenotypes was described, and interspecific progenies were then examined and graded according to the number of individuals falling into each phenotypic category. This method lacks precision and introduces an a priori bias by imposing arbitrary categories upon the observed distributions. Quantitative measurements of stigma dimensions, being more precise and free of preconceptions, are better suited to characterize both continuous and discontinuous distributions.

An example of genetic analysis of stigma inheritance based on quantitative data is the work of Grant (1950), involving hybrids between the subspecies *Gilia capitata capitata* (Polemoniaceae), with short stigmas,

and _G. c. Chamissonis_, with long stigmas. Stigmas of parental species, F_1 hybrids and segregating generations were measured to the nearest tenth of a millimeter. The recovery of one parental stigma type among 176 F_2 progeny indicated that 3 to 5 genes determine the difference in stigma length in these two subspecies. Stigma length in the F_1 hybrid was not intermediate, but approached that of _G. c. capitata_, the parent with short stigmas. The F_2 and backcross progenies were also skewed toward the shorter parent. Based on these data, Grant proposed that stigma length in _G. capitata_ is governed by approximately four genes, one of which is a major gene exhibiting dominance, while the others are modifiers of equal effect.

MATERIALS AND METHODS

Plant materials employed as parental lines in this study are listed in Table 1, together with corresponding abbreviations, if any, used in tables and graphs. All *P. vulgaris* lines and cultivars are determinate, bush types, with the exception of the half-runner breeding line 7-1404. All *P. coccineus* PI lines are indeterminate vines. 'Hammond's Dwarf White' is a determinate *P. coccineus* cultivar.

All crosses between fertile parental materials were made using emasculated flower buds at the stage one day prior to anthesis. A pair of honed forceps was used to carefully open plump buds, and the left wing petal was usually removed to provide better access to the keel. Grasping the lip of the keel aperature with the forceps, a portion of the keel was gently peeled back and away to reveal the reproductive structures within. The anthers were removed with the aid of the forceps, taking special care to avoid injuring the style. After emasculation, the stigma was inspected with a magnifying lens to ensure that the surface was free of pollen. Cross-pollination followed immediately. From the pollen parent, the tip of a style was taken from a freshly opened flower, bearing with it a load of pollen trapped in the stylar hairs. Cross-pollination was effected by rubbing the pollen brush from the male source across the surface of the emasculated bud. The petals of the opened bud were reclosed to protect the reproductive parts, and the bud was tagged with the date and pollen

Table 1. List of plant materials used in study of stigma shape inheritance in *Phaseolus vulgaris* X *Phaseolus coccineus* hybrids.

Phaseolus vulgaris	*Phaseolus coccineus*
Cultivars	Cultivars
'Swiss'	'Hammond's Dwarf White' ('HDW')
'Harvester' ('Harv.')	Plant Introduction Lines
'Sprite'	PI 273666
'Light Red Kidney' ('LRK')	PI 321088
Intraspecific F_1 Hybrid	PI 311819
'Light Red Kidney' X 'Oregon State U. 58' ('LRK' X 'OSU 58')	PI 312009
Breeding Lines	
F_3-1	
7-1404	
6-19	

source. Following a suggestion by Ibrahim and Coyne (1975), in crosses involving P. coccineus as the seed parent, White's nutrient solution was applied to stigmas with a small brush before pollination. Use of this technique to enhance fertility of selfed P. vulgaris X P. coccineus F_1 hybrids was found not to be effective. In some interspecific F_1 hybrids, fertility was so poor that they could serve as seed parents without emasculation. Such was the case with the 'Swiss' X 'Hammond's Dwarf' F_1 hybrid used as female parent in the backcross to the P. vulgaris intraspecific hybrid 'Light Red Kidney' X 'OSU 58'. Since bean flowers begin to degenerate within a day of the time of bud opening, most crosses were made in the morning hours when pollen and flowers were fresh, and work late in the day was avoided.

With few, unimportant exceptions, the early interspecific generations and inbred progenies through the F_5 generation were grown in pots in greenhouses during the fall and winter months of 1976-1977, 1977-1978, and 1978-1979. Insectproof greenhouses were used to raise inbred progenies being tested for outcrossing flower structure, although insect pollinators were not a problem in any of the greenhouses during the winter months. Large, segregating populations, such as the BC-F_2 generations resulting from the backcross of selected interspecific F_2, F_3, and F_4 plants to P. vulgaris cultivars, were grown in the field in the late spring of 1977, 1978, and 1979, and in the early fall of 1978.

Flowers were collected in the morning or early afternoon on the day of anthesis in order to obtain stigmas undamaged by insect pollinators or normal deterioration. Mature flower buds were sometimes

collected one day prior to anthesis from materials in which heavy pollen shed tended to obscure the stigma surface at anthesis, or when insect pollinators were particularly active, as in the spring field plantings. Flowers collected in the field or greenhouse were placed in paper bags labelled by individual plant and transported to the laboratory for microscopic measurement of the stigmas. Total elapsed time from collection of flowers to measurement of stigmas did not exceed 3 hours.

All stigma observations were made with the aid of a Bausch & Lomb Stereozoom dissecting microscope at 25x magnification against a dark background with side illumination. Measurements of stigma length were made with an ocular reticle calibrated at 25x with a stage micrometer. The terminal portion (about .5mm) of style with stigma attached was removed from the keel petals with forceps and placed on a petrie dish cover which could be freely rotated on the microscope stage. By this arrangement, the axis of the style could be readily positioned to coincide with the linear scale of the ocular reticle, thus facilitating stigma measurement.

Two different stigma dimensions were measured--the length of the internal surface of the stigma (Int) and the length of the external surface of the stigma (Ext) (Fig. 3). In the great majority of materials observed in this study, these measurements were straightforward and unambiguous. However, two kinds of exceptions were noted, both involving deformations of the stylar axis of orientation. One kind of problem arose in measuring stigma lengths of _P_. _coccineus_ or progenies of interspecific materials backcrossed to _P_. _coccineus_. The

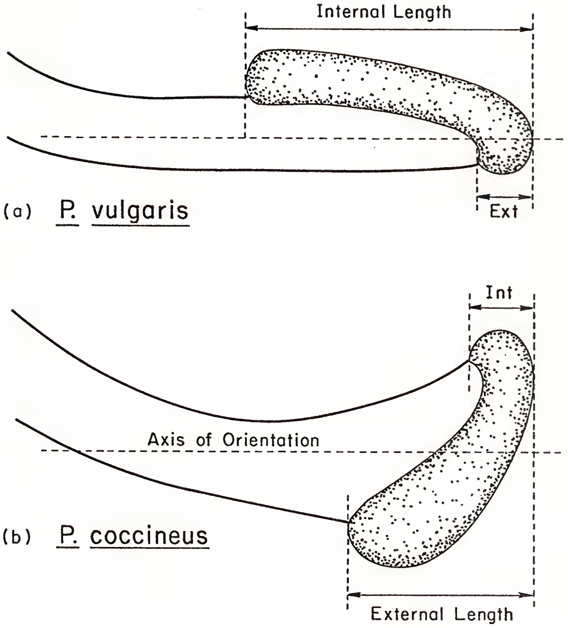


Figure 3. Orientation of styles of Phaseolus vulgaris and Phaseolus coccineus for measurement of internal (Int) and external (Ext) stigma length. (a) Stylar tip of P. vulgaris is linear, facilitating orientation and reproducible measurement. (b) Stylar tip of P. coccineus has no obvious axis of orientation, and measurement of stigmas is less precise.

nearly co-linear arrangement of stylar axis and stigma surface which facilitates measurement in P. vulgaris (Fig. 3(a)) does not exist in P. coccineus, nor in segregating interspecific materials resembling P. coccineus. There is no obvious, correct way to orient the style in the latter materials, since the style is curved in the region of the stigma, and the internal and external margins of the style seem to follow different arcs (Fig. 3(b)). The approach adopted here has been to take a median course, aligning neither the internal nor the external margin of the style with the axis of orientation. Rather the style was positioned so that the axis of orientation bisected the acute angle formed by the different arcs of the internal and external margins of the style in the region of the stigma. This kind of measurement necessarily loses some precision.

Another problem occurring occasionally in interspecific progenies involves deformation of the style either by torsion about the axis or by bending of the style tip in the external direction. Such aberrations are rare and usually vary within individual plants, so that a second sampling overcomes the problem. The point to be made here is that the style axis is the key to accurate, repeatable stigma measurement, and any deformation that disturbs the orientation of the stigma with respect to the stylar axis will complicate measurement and reduce accuracy.

Figure 4 illustrates in schematic form some of the stigma types observed in the course of this study. Stigma 4(a) represents P. vulgaris, while 4(e) is the P. coccineus stigma. The F_1 interspecific hybrids have stigmas similar to 4(b) and are characterized

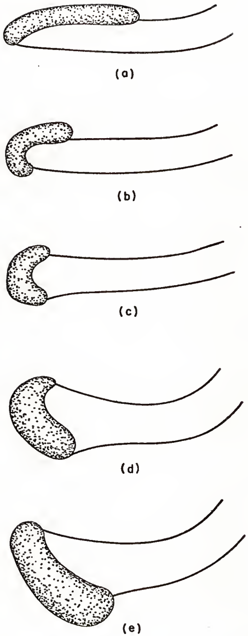


Figure 4. Some representative stigma types of Phaseolus vulgaris, Phaseolus coccineus and materials derived from hybridization. (a) P. vulgaris. (b) Stigma typical of P. vulgaris X P. coccineus F_1 hybrid. (c) Terminal stigma type observed in F_2 and higher inbred generations. (d) Extrorse stigma type observed in progenies resulting from backcrossing to P. coccineus and also occasionally in interspecific F_2 populations. (e) P. coccineus.

by a shorter internal extent and a more pronounced external extent than stigmas of P. vulgaris. The terminal stigma type, 4(c), has internal and external surfaces of equal extent. Stigma 4(d) has a greater external extent than internal extent and shows some of the stylar characteristics of P. coccineus.

Measurements of several different stigmas from each plant were averaged to obtain an estimate of the individual genotypic expression. Averages based on two to five, or more, stigma observations per plant were calculated for different populations, depending primarily upon the amount of time available for data collection.

Selection of interspecific materials to be utilized in inbreeding and backcrossing experiments was based on two criteria. One of these was stigma type, in accordance with the goal of the study--to transfer the extrorse stigma type of P. coccineus into P. vulgaris germplasm in order to enhance outcrossing ability. In some of the earliest interspecific progenies produced, only internal stigma length was recorded. These progenies were the F_1 generation, the backcross of the F_1 to P. vulgaris, and the F_2 generation derived from the cross of P. vulgaris 'Swiss' X P. coccineus 'Hammond's Dwarf White' and the F_1 population obtained from P. vulgaris 'Harvester' X P. coccineus PI line crosses. Individual plants from the 'Swiss' X 'HDW' F_2 population were selected for backcrossing to P. vulgaris on the basis of internal stigma length. Only plants with internal stigma surfaces shorter than 0.60mm were included in the backcross program. In all materials subsequently employed or produced, both internal and external stigma lengths were measured. With the added information

regarding external stigma length, it became convenient to quantify various stigma types in terms of the ratio of the external stigma length to the internal stigma length (Ext/Int). In inbreeding interspecific materials from F_2 to F_4, selection against parents with small stigma ratios (introrse stigmas) was not severe, since it was desired to observe segregation in F_3 and F_4 progenies derived from parents differing widely in stigma stypes. However, in selecting interspecific inbreds for backcrossing to P. vulgaris, plants with large Ext/Int ratios were favored, since these seemed most likely to succeed in transferring stigma "externalizing" factors into P. vulgaris germplasm. Plants with Ext/Int ratios less than 0.70 were selected against, unless they possessed other redeeming characters, such as a useful degree of fertility.

Use of the Ext/Int ratio as a selection criterion has some limitations. Stigmas with the same Ext/Int ratio may differ considerably in the absolute lengths of internal and external surfaces, a fact which may be important in terms of the relationship between internal stigma length and anther position. Another consequence is that identical Ext/Int ratios do not necessarily imply similar genotypes.

In order to test the outcrossing ability of an individual in a population segregating for stigma type, it is necessary to protect the plant from insect pollinators, so that the degree to which it is capable of self-pollination can be determined. At the same time, some flowers must be artificially hand-selfed to test for fertility. Plants which produce seed spontaneously when isolated from insects are both fertile and self-pollinating. Isolated plants which produce

seed from manually selfed flowers, but not otherwise, merit investigation as outcrossing types. Plants which produce little or no seed of any kind have sterility problems, and the importance of stigma type with respect to outcrossing ability cannot be adequately evaluated in such materials. Unfortunately, much of the *P. vulgaris* X *P. coccineus* interspecific material falls into the third category. The reduction in fertility accompanying hybridization necessitates that fertility be another selection criterion in breeding for outcrossing behavior.

In this study, fertility was quantified according to the following formula:

$$\text{Fertility} = \frac{(\#\text{ of seeds produced by manual self-pollination})^2}{\#\text{ of manual self-pollinations}}$$

Testing of inbred plants for fertility was carried out in screened greenhouses to exclude insects. Six flowers on each plant were manually self-pollinated. The number of self-pollinations per plant was intentionally kept low in order to avoid a false indication of sterility due to overtaxing the reproductive ability of the rather unthrifty hybrid materials. The fertility index was calculated for each plant and considered along with the Ext/Int stigma ratio when making selections for further breeding efforts. Volunteer seed on hybrid materials was rare.

In large, field-grown BC-F_2 populations, segregation for extrorse stigma types was another rare event. In order to locate such individuals, the bulk of the planting was surveyed, and plants were qualitatively evaluated for stigma type with the aid of a hand lens. Plants with the most extrorse stigmas, amounting to between 1% and 7.5% of

the surveyed population were flagged and stigma measurements were obtained. On the basis of the Ext/Int ratio calculated from stigma measurements, the most extrorse individuals were identified and enclosed in small, transportable, screened cages in the field. The caged plants were then selfed to saturation to ensure continuation of the line, and the fertility index was calculated as above. Plants with the most extrorse stigmas and the best levels of fertility were marked for further backcrossing.

In attempting to portray the results of this study in graphic form, it became apparent that the Ext/Int stigma ratio introduced a serious distortion when used as a scale upon which to plot stigma type frequencies. The Ext/Int ratio varies in a non-linear fashion and tends to understate differences between introrse stigma types, while overemphasizing differences between extrorse stigma types. Data transformations which eliminate this distortion also make the message of the graphed data obscure. Stigma shape relationships are portrayed most faithfully in graphic form by simply plotting external stigma length against internal stigma length in a two-dimensional scatter diagram. Progeny means can be calculated for such two-dimensional, bivariate distributions, and it is also possible to calculate statistics equivalent to univariate variance and confidence limits. The dispersion of points in a scatter diagram is a measure of bivariate variance. It is obtained by calculating the determinant of the population covariance matrix, $|S|$, according to the following formula:

$$|S| = s_x^2 \, s_y^2 - s_{xy}^2$$

where s_x^2 and s_y^2 are the variances and s_{xy} is the covariance of the internal and external stigma lengths in a plant population. The $|S|$ statistic has been used in the present study as an indication of stigma shape heterogeneity in progenies, and by inference, as a measure of homozygosity for stigma genes. Since the numerical value of $|S|$ is usually a very small fraction, graph axes indicating this variable are graduated in units of $|S| \times 10^6$.

Confidence regions can be described about the means of bivariate distributions just as confidence limits can be calculated for univariate variables. Since scatter diagrams of stigma types are based on two variables--external stigma length and internal stigma length--the confidence region will be an ellipse. The shape of the ellipse is a function of the correlation between the variables, and the size or area of the ellipse is a function of the confidence coefficient, $1 - \alpha$. In the present work, use has been made of equal frequency ellipses for observations, that is, ellipses in which the probability of obtaining an observation equal to, or farther from, the population mean is the same, α, for all points on the ellipse. The procedure for calculating equal frequency ellipses is outlined by Sokal and Rohlf (1969). A 95% confidence region described about the mean stigma type of a homozygous parental line or advanced generation hybrid progeny sets an approximate boundary for individuals of that genotype. In continuously distributed BC and F_2 populations, the number of individuals with stigma types falling within the parental confidence regions gives an estimate of the proportion of parental genotypes recovered in the segregating progeny. This information may then be used to estimate the number of genes controlling segregation.

The estimate of narrow sense heritability for stigma shape obtained in this work is based upon the correlation between F_3 progeny means and their respective F_2 parent values. This procedure yields the same result as that obtained by regressing F_3 progeny means, coded in terms of standard deviation units, upon similarly coded stigma data for F_2 parents (Frey and Horner, 1957). Graphs of regressions in the text are performed on uncoded data, and hence the regression and correlation coefficients differ. To compensate for the effect of inbreeding due to self-pollination of the F_2 parents, the correlation coefficient was multiplied by a factor of 2/3 to give the heritability estimate (Smith and Kinman, 1965).

RESULTS AND DISCUSSION

Influence of Environment on Stability of Stigma Expression

In order to ascertain the importance of environmental variability in determining expression of the gene(s) controlling stigma length, two types of experiments were conducted. In the first, individual F_2 and F_3 plants, derived from P. vulgaris 'Harvester' X P. coccineus PI line crosses were used. Samples consisting of 4 stigmas were collected from each plant twice during the flowering period, and differences between sample stigma length means within individual plants were tested for significance. Results of t tests are given in Table 2.

In the second experiment, stigma lengths of two common bean cultivars, 'Harvester' and 'Sprite', were measured under different environmental conditions. One plot of each cultivar, consisting of 8 to 10 plants, was grown under greenhouse conditions in the fall, 1977, while a second plot was planted in the field during the spring of 1980. Several stigmas, ranging from 2 to 10, were measured on each plant. The significance of mean stigma length differences, attributable to environmental differences, was tested within cultivars by analysis of variance. The results are presented in Table 3.

Table 2 demonstrates that for 22 of the 32 plants in the experiment, changes in environment between sampling dates did not significantly affect mean stigma length. In the remaining 10 plants, either internal or external stigma length means differed significantly between sampling dates, but

Table 2. Stability of stigma length expression over time in F_2 and F_3 individuals derived from <u>Phaseolus vulgaris</u> 'Harvester' X <u>Phaseolus coccineus</u> PI line crosses. Stability of expression was tested by determining the significance of the difference in stigma length sample means (n = 4) calculated for the same plant at 2 different times during the flowering period.

		INTERNAL STIGMA LENGTH		EXTERNAL STIGMA LENGTH	
Plant Number	Sample	Sample Mean (mm)	t	Sample Mean (mm)	t
F_2 $\overline{66}$-5	1	0.670 ± 0.038	n.s.	0.330 ± 0.012	n.s.
	2	0.690 ± 0.038		0.310 ± 0.012	
F_2 $\overline{66}$-15	1	0.645 ± 0.019	n.s.	0.400 ± 0.028	*
	2	0.650 ± 0.020		0.360 ± 0.001	
F_2 $\overline{88}$-7	1	0.450 ± 0.090	n.s.	0.540 ± 0.023	n.s.
	2	0.465 ± 0.041		0.520 ± 0.016	
F_2 $\overline{09}$-3	1	0.520 ± 0.016	n.s.	0.450 ± 0.038	n.s.
	2	0.565 ± 0.034		0.485 ± 0.025	
F_2 $\overline{09}$-14	1	0.650 ± 0.026	n.s.	0.310 ± 0.020	n.s.
	2	0.670 ± 0.026		0.310 ± 0.026	
F_2 $\overline{09}$-20	1	0.650 ± 0.035	n.s.	0.410 ± 0.038	*
	2	0.625 ± 0.019		0.355 ± 0.010	
F_2 $\overline{09}$-32	1	0.480 ± 0.033	n.s.	0.455 ± 0.019	*
	2	0.520 ± 0.057		0.425 ± 0.010	
F_2 $\overline{09}$-42	1	0.545 ± 0.034	n.s.	0.415 ± 0.019	n.s.
	2	0.560 ± 0.046		0.385 ± 0.044	
F_2 $\overline{09}$-49	1	0.580 ± 0.037	n.s.	0.340 ± 0.016	n.s.
	2	0.570 ± 0.048		0.335 ± 0.010	
F_2 $\overline{19}$-21	1	0.625 ± 0.019	n.s.	0.390 ± 0.026	n.s.
	2	0.620 ± 0.016		0.375 ± 0.025	
F_2 $\overline{19}$-30	1	0.490 ± 0.035	n.s.	0.360 ± 0.028	n.s.
	2	0.515 ± 0.010		0.335 ± 0.019	
F_3 66-7-5	1	0.460 ± 0.023	n.s.	0.380 ± 0.016	*
	2	0.540 ± 0.085		0.425 ± 0.019	
F_3 66-7-9	1	0.740 ± 0.023	**	0.290 ± 0.026	n.s.
	2	0.790 ± 0.012		0.280 ± 0.016	
F_3 66-18-2	1	0.790 ± 0.026	n.s.	0.310 ± 0.026	n.s.
	2	0.770 ± 0.012		0.315 ± 0.019	

Table 2. Continued

		INTERNAL STIGMA LENGTH		EXTERNAL STIGMA LENGTH	
Plant Number	Sample	Sample Mean (mm)	t	Sample Mean (mm)	t
F_3 66-18-7	1	0.575 ± 0.019	n.s.	0.270 ± 0.020	n.s.
	2	0.610 ± 0.026		0.285 ± 0.010	
F_3 66-18-8	1	0.735 ± 0.019	n.s.	0.280 ± 0.001	n.s.
	2	0.740 ± 0.016		0.300 ± 0.016	
F_3 66-24-4	1	0.530 ± 0.038	n.s.	0.495 ± 0.030	n.s.
	2	0.520 ± 0.065		0.500 ± 0.023	
F_3 66-24-10	1	0.760 ± 0.001	*	0.305 ± 0.019	n.s.
	2	0.775 ± 0.010		0.325 ± 0.019	
F_3 88-1-3	1	0.770 ± 0.020	n.s.	0.285 ± 0.025	n.s.
	2	0.795 ± 0.019		0.305 ± 0.019	
F_3 88-2-4	1	0.460 ± 0.069	n.s.	0.495 ± 0.025	n.s.
	2	0.470 ± 0.038		0.520 ± 0.033	
F_3 88-4-4	1	0.735 ± 0.019	n.s.	0.340 ± 0.023	n.s.
	2	0.740 ± 0.016		0.375 ± 0.019	
F_3 88-4-5	1	0.870 ± 0.020	n.s.	0.235 ± 0.010	n.s.
	2	0.880 ± 0.057		0.245 ± 0.010	
F_3 88-4-8	1	0.770 ± 0.026	n.s.	0.350 ± 0.020	n.s.
	2	0.815 ± 0.050		0.370 ± 0.012	
F_3 88-4-9	1	0.705 ± 0.030	n.s.	0.350 ± 0.026	*
	2	0.745 ± 0.019		0.385 ± 0.019	
F_3 88-6-6	1	0.595 ± 0.057	n.s.	0.425 ± 0.019	n.s.
	2	0.590 ± 0.060		0.430 ± 0.020	
F_3 88-14-3	1	0.495 ± 0.030	n.s.	0.430 ± 0.020	*
	2	0.490 ± 0.012		0.500 ± 0.037	
F_3 09-5-1	1	0.715 ± 0.025	n.s.	0.370 ± 0.020	*
	2	0.705 ± 0.019		0.405 ± 0.010	
F_3 09-5-2	1	0.740 ± 0.101	n.s.	0.390 ± 0.050	n.s.
	2	0.745 ± 0.019		0.420 ± 0.023	
F_3 09-8-5	1	0.600 ± 0.016	*	0.415 ± 0.030	n.s.
	2	0.670 ± 0.035		0.440 ± 0.033	

Table 2. Continued

		INTERNAL STIGMA LENGTH		EXTERNAL STIGMA LENGTH	
Plant Number	Sample	Sample Mean (mm)	t	Sample Mean (mm)	t
F_3 09-14-4	1	0.770 ± 0.050	n.s.	0.340 ± 0.043	n.s.
	2	0.735 ± 0.019		0.360 ± 0.016	
F_3 09-14-8	1	0.730 ± 0.048	n.s.	0.405 ± 0.010	n.s.
	2	0.700 ± 0.016		0.400 ± 0.016	
F_3 19-7-1	1	0.735 ± 0.019	n.s.	0.380 ± 0.046	n.s.
	2	0.720 ± 0.037		0.395 ± 0.010	

* Significant at the 5% level.

** Significant at the 1% level.

Table 3. Stability of stigma length expression over environments in Phaseolus vulgaris 'Harvester' and 'Sprite'. Stability of expression was tested by performing an analysis of variance of stigma length for each cultivar grown under 2 different environments (greenhouse--fall, 1977, and field--spring, 1980).

P. vulgaris 'Harvester'

	INTERNAL STIGMA LENGTH				EXTERNAL STIGMA LENGTH			
Source of Variation	SS	df	MS	F	SS	df	MS	F
Between environments	0.2713	1	0.2713	1.1796 n.s.	0.0001	1	0.0001	0.0003 n.s.
Among plants within environments	4.1397	18	0.2300		0.0061	18	0.0003	
Among measurements within plants	0.0285	50						
Total	4.4395	69						

P. vulgaris 'Sprite'

	INTERNAL STIGMA LENGTH				EXTERNAL STIGMA LENGTH			
Source of Variation	SS	df	MS	F	SS	df	MS	F
Between environments	0.0019	1	0.0019	0.5758 n.s.	0.0021	1	0.0021	6.3444 *
Among plants within environments	0.532	16	0.0033		0.0053	16	0.0003	
Among measurements within plants	0.0345	44						
Total	0.0896	61						

		INTERNAL STIGMA LENGTH		EXTERNAL STIGMA LENGTH	
Cultivar	Environment	Mean (mm)	F	Mean (mm)	F
'Harvester'	Grnhse-Fall '77	1.040	1.1796	0.206	0.3333
	Field-Spring '80	1.008	n.s.	0.203	n.s.
'Sprite'	Grnhse-Fall '80	0.987	0.5758	0.204	6.3444
	Field-Spring '80	0.970	n.s.	0.218	*

* Significant at the 5% level.

in no case were both internal and external means significantly different for the same plant. In all cases except one, significance was at the 5% level, the exception being significant at the 1% level. Even though stigma lengths in 10 plants were significantly affected by environmental changes during the flowering period, the maximum observed difference between paired means was only 16.3% of the smaller mean stigma length value.

Table 3 indicates that the mean stigma length of P. vulgaris 'Harvester' was not significantly affected by a combination of location and seasonal differences. The same was true for the mean internal stigma length of 'Sprite'. The mean external stigma length of 'Sprite' differed significantly at the 5% level, but the difference between external means was only 6.9% of the smaller mean value.

It would be logical to suppose that metrical characters having a critical role in the reproduction of an organism, as does stigma length in the common bean, may be well buffered against environmental variation, so that reproduction may proceed without interruption under a broad range of environmental conditions. The results reported in Tables 2 and 3 suggest that the expression of the stigma gene(s) in P. vulgaris and in P. vulgaris X P. coccineus hybrids is quite stable in the face of environmental variation. Furthermore, environmental effects of the magnitude observed, even when statistically significant, would not be likely to cause a plant to be misclassified with regard to stigma type. Consequently, in the execution of this study a mean stigma length value calculated at any point in the flowering period of a plant was judged to accurately represent the genotype. Also, stigma length means obtained in different seasons or from different locations were considered to be directly comparable, and no effort was made to monitor control populations.

Stigma Inheritance in Early Generations Following Interspecific Hybridization

Not all P. vulgaris X P. coccineus hybridizations attempted in this study were successful. The cross involving P. vulgaris 'Sprite' X P. coccineus 'Hammond's Dwarf White' produced plump F_1 seed of normal appearance, but F_1 plants were uniformly of the B-dwarf type described by Kedar and Bemis (1960), and they did not survive beyond the seedling stage. The F_1 progenies of P. vulgaris 'Swiss' X P. coccineus 'Hammond's Dwarf White' and P. vulgaris 'Harvester' X P. coccineus PI lines were fully viable. The F_1 generation of the former cross consisted of determinant, bush plants, while the latter cross produced vigorous F_1 progenies with uniformly indeterminant, vining growth.

Frequency distributions of internal stigma lengths for parents and hybrid generations resulting from P. vulgaris 'Swiss' X P. coccineus 'Hammond's Dwarf White' and P. vulgaris 'Harvester' X P. coccineus PI lines are given in histograms in Figs. 5 and 6. All parental materials and F_1 hybrid populations have small standard deviations relative to the standard deviations of F_2 and backcrossed generations. Coefficients of variability for parental and F_1 populations ranged from 3.5% to 6.2%. Stigma measurements of P. coccineus parental materials were recorded only for 'Hammond's Dwarf White' and PI 273666. The F_1 generations of these two P. coccineus parents, produced by crossing with P. vulgaris 'Swiss' and 'Harvester', respectively, are displaced somewhat from the midparent values (M) toward the P. vulgaris parent. In the cross involving P. vulgaris 'Swiss' X P. coccineus 'Hammond's Dwarf White', the deviation from the midparent value amounts to 18.0% of the difference in internal stigma length between the midparent and the 'Swiss' mean. In the P. vulgaris 'Harvester' X P.

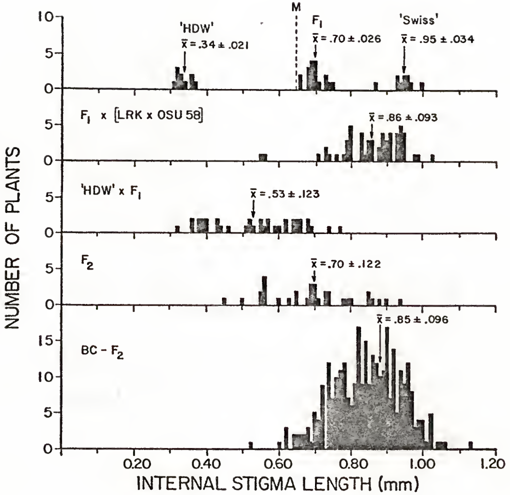


Figure 5. A comparison of internal stigma length distributions, with means and standard deviations, in Phaseolus vulgaris 'Swiss', Phaseolus coccineus 'Hammond's Dwarf White' ('HDW'), the F_1 interspecific hybrid ('Swiss' ♀), the backcross of the interspecific F_1 to the P. vulgaris hybrid 'Light Red Kidney' X 'Oregon State U. 58', the BC-F_2 generation produced by selfing members of the last-mentioned population, the backcross of the interspecific F_1 to P. coccineus 'Hammonds's Dwarf White', and the F_2 generation. The internal stigma length value of the midparent is designated by M.

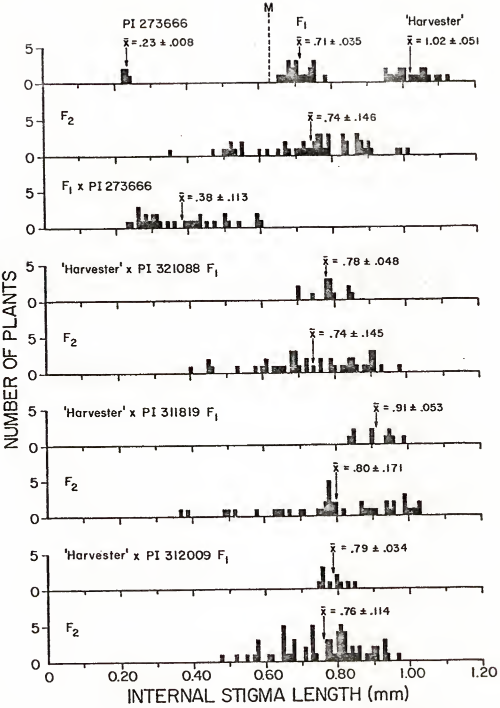


Figure 6. A comparison of internal stigma length distributions, with means and standard deviations, in F_1, F_2 and backcross progenies derived from crosses of Phaseolus vulgaris 'Harvester' with 4 Phaseolus coccineus PI lines--PI 273666, PI 321088, PI 311819 and PI 312009. The internal stigma length value of the mid-parent is designated by M.

coccineus PI 273666 hybrid, the corresponding deviation amounts to 21.5%. There is some variation in means of F_1 populations produced by crossing P. vulgaris 'Harvester' with different P. coccineus PI lines, reflecting differences in the genetic constitutions of the P. coccineus parental materials. This was particularly evident in the cross involving PI 311819, in which the mean of the F_1 generation falls so close to the P. vulgaris 'Harvester' mean, that the internal stigma lengths of some individuals overlap the parental P. vulgaris range.

The F_2 generations are much more variable than the F_1 generations, having coefficients of variability ranging from 15.4% to 21.3%. Most F_2 populations were centered on or near the mean of the corresponding F_1 population, with the exception of the F_2 produced from the cross of P. vulgaris 'Harvester' X P. coccineus PI 311819. The mean of this F_2 population was 0.11mm shorter than the F_1 mean, indicating that the relatively large mean length of the F_1 population masked a great deal of variability for shorter internal stigma lengths which reappeared in F_2 segregants.

The scatter plots of internal stigma length versus external stigma length for F_2 populations of crosses of P. vulgaris 'Harvester' X P. coccineus PI lines, shown in Fig. 7 and Fig. 8, reveal that 3 of the 4 populations were too small to recover the parental types. The F_2 progenies derived from crosses with PI 273666, PI 321088, and PI 312009 contained a total of 159 plants, none of which possessed parental stigma types. This suggests that 3 or more genes are involved in control of stigma shape in these materials. The exception was the cross involving PI 311819, which segregated for 3 or 4 P. vulgaris stigma types in an

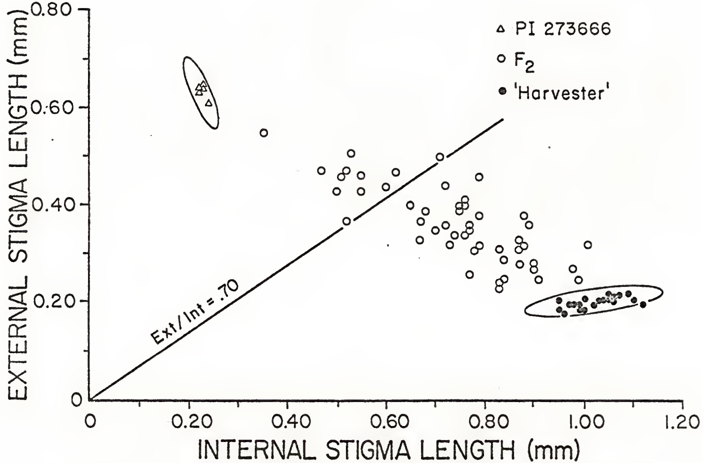
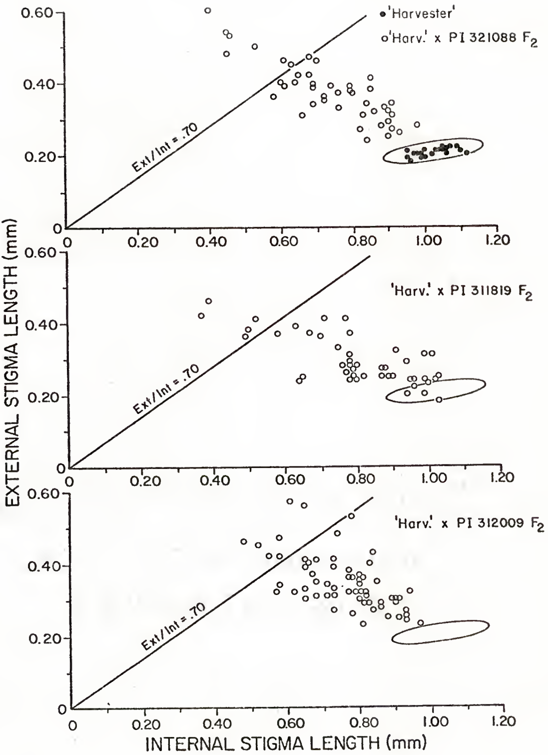


Figure 7. Distribution of stigma types in an F_2 population produced by crossing Phaseolus vulgaris 'Harvester' X Phaseolus coccineus PI 273666. Ellipses represent 95% confidence regions for populations upon which they are centered.

Figure 8. Distribution of stigma types in 3 interspecific F_2 populations produced by crossing Phaseolus vulgaris 'Harvester' X Phaseolus coccineus PI lines. The ellipses represent the 95% confidence region for 'Harvester'.



• 'Harvester'
o 'Harv.' x PI 321088 F2
'Harv.' x PI 311819 F2
'Harv.' x PI 312009 F2
Ext/Int = .70
EXTERNAL STIGMA LENGTH (mm)
INTERNAL STIGMA LENGTH (mm)
0.60
0.40
0.20
0
0.20
0.40
0.60
0.80
1.00
1.20

F_2 consisting of 64 individuals. This result suggested that only 2 genes control stigma shape in the cross involving PI 311819.

While distributions of internal stigma length appear in the histograms to be continuous for all F_2 populations, earlier experimental results (Table 4) were classified for qualitative differences based on the appearance of the stigma when both internal and external stigma lengths were considered. From these observations, it was concluded that major gene segregation was occurring in the F_2 generation of _P. vulgaris_ 'Swiss' X _P. coccineus_ 'Hammond's Dwarf White'. The observed ratio was a good fit to that expected from a 2-gene system with dominant epistasis. Hypothetical genotypes associated with the observed stigma classes are listed in Table 5. Variation within the introrse stigma class was continuous, ranging from an internal stigma length of just 0.55mm, which overlapped the terminal class, to 0.94mm, which fell within the parental _P. vulgaris_ range. This continuous distribution of introrse stigma types was attributed to the effect that modifying factors, contributed from the _P. coccineus_ genome, had upon the _P. vulgaris_ alleles at the major gene loci.

The 2-gene, dominant epistasis model was found to be less satisfactory in explaining segregation in the larger F_2 populations derived from _P. vulgaris_ 'Harvester' X _P. coccineus_ PI lines (Table 6). Difficulty was also experienced in defining the boundary between the terminal class and the short end of the variable introrse class, which frequently seemed to overlap. This led to the adoption of a somewhat arbitrary definition of the terminal stigma as one having an Ext/Int ratio equal to, or greater than,0.70. In spite of the fact that this created a generous lower limit for the terminal class, terminal stigmas were generally deficient in the observed F_2 populations. However, a shortage of extrorse stigma types

Table 4. Segregation for stigma type in a *Phaseolus vulgaris* 'Swiss' X *Phaseolus coccineus* 'Hammond's Dwarf White' F_2 population tested for goodness of fit to a 12:3:1 ratio by chi-square analysis.

	Number of Plants			
	Total	Introrse	Terminal	Extrorse
Observed	34	27	5	2
Expected		25.5	6.4	2.1

χ^2 (12:3:1) = 0.399

P at 2 d.f. = 0.90 - 0.75

Table 5. Theoretical segregation for stigma type in a Phaseolus vulgaris X Phaseolus coccineus F_2 generation, based on a 2-gene, dominant epistasis model of inheritance.

Frequency	Genotype	Frequency	Phenotype
1/16	AABB	12/16	Introrse
2/16	AABb		
1/16	AAbb		
2/16	AaBB		
4/16	AaBb		
2/16	Aabb		
1/16	aaBB	3/16	Terminal
2/16	aaBb		
1/16	aabb	1/16	Extrorse

Table 6. Observed segregation for stigma type in 4 _Phaseolus vulgaris_ 'Harvester' X _Phaseolus coccineus_ PI line F_2 populations tested for goodness of fit to a 12:3:1 ratio by chi-square analysis.

	Number of Plants					
	Total	Introrse	Terminal	Extrorse	χ^2(12:3:1)	P
'Harv.' X PI 273666 F_2	50	38	11	1	1.734	0.50-0.25
'Harv.' X PI 321088 F_2	44	37	6	1	2.213	0.50-0.25
'Harv.' X PI 311819 F_2	45	40	5	0	5.370	0.10-0.05
'Harv.' X PI 312009 F_2	64	57	7	0	7.771	0.025-0.01
Pooled	203	172	29	2	13.723	0.005-0.001
Heterogeniety					3.365	0.90-0.75

was the chief reason for the poor fit of the proposed model in the case of the F_2 populations derived from P. vulgaris 'Harvester' X P. coccineus PI line crosses. The attenuated extrorse tails of the F_2 frequency distributions were reflected statistically in a moderate negative skew, significant only in the cross involving PI 311819 (Table 7). These facts suggest that P. coccineus stigma genes may have been selectively eliminated in the F_2 generations of these crosses through formation of inviable or poorly competitive gametes or zygotes. Support for this notion was found in the observation that another P. coccineus character with a known inheritance also appeared much less frequently than expected in the F_2 populations derived from P. vulgaris 'Harvester' X P. coccineus PI line crosses. The indeterminate, vining growth habit of the parental P. coccineus PI lines is monofactorially inherited and was completely dominant in the F_1 interspecific hybrid populations. The frequency of vining plants in the F_2 populations was drastically reduced from the expected 75% (Table 8). This is best explained on the basis of selective elimination of gametes or zygotes bearing the gene for indeterminate growth or closely linked, deleterious genes.

The backcross generation produced by crossing the interspecific F_1 hybrid of P. vulgaris 'Swiss' X P. coccineus 'Hammond's Dwarf White' with the intraspecific hybrid P. vulgaris 'Light Red Kidney' X P. vulgaris 'Oregon State U. 58' was distributed continuously with regard to internal stigma length between the mean of the interspecific F_1 and the probable region of the intraspecific mean (Fig. 5). The latter is not known precisely, since it was not measured microscopically, but the internal stigma length appeared to the unaided eye to be similar to other P. vulgaris cultivars, and was assumed to be approximately 1.00mm. Two plants,

Table 7. Test of skewing in the distribution of internal stigma lengths (Int) in F_2 and backcrossed populations derived from the interspecific crosses _Phaseolus vulgaris_ 'Swiss' X _Phaseolus coccineus_ 'Hammond's Dwarf White' and _P. vulgaris_ 'Harvester' X _P. coccineus_ PI lines.

Population	n	$\overline{Int}$ (mm)	std. dev.(s)	$\sum \frac{(Int-\overline{Int})^3}{n} / s^3$	
'Swiss' X 'HDW' F_2	33	0.70	0.122	0.0566	n.s.
F_1 X ('LRK' X 'OSU 58')	54	0.87	0.073	0.2513	n.s.
'HDW' X F_1	37	0.53	0.123	0.1022	n.s.
'Harv.' X PI 273666 F_2	50	0.74	0.146	- 0.4675	n.s.
F_1 X PI 273666	31	0.38	0.113	0.5139	n.s.
'Harv.' X PI 321088 F_2	45	0.74	0.145	- 0.5304	n.s.
'Harv.' X PI 311819 F_2	45	0.80	0.171	- 0.7138	*
'Harv.' X PI 312009 F_2	64	0.76	0.114	- 0.3642	n.s.

* Significant at the 5% level.

Table 8. Observed segregation for bush or vine habit in Phaseolus vulgaris 'Harvester' X Phaseolus coccineus PI line F_2 populations tested for goodness of fit to a 3:1 ratio by chi-square analysis.

	Number of Plants				
	Total	Vine	Bush	χ^2	P
'Harv.' X PI 273666 F_2	50	8	42	92.827	<0.001
'Harv.' X PI 321088 F_2	44	2	42	116.485	<0.001
'Harv.' X PI 311819 F_2	45	2	43	119.474	<0.001
'Harv.' X PI 312009 F_2	64	8	56	133.000	<0.001
Pooled	203	20	183	459.509	<0.001
Heterogeneity				2.277	0.75-0.50

BC 4-22 and BC 2-15, with short stigma lengths of about 0.55mm do not fall in the continuous distribution, but lie considerably below it. These plants are almost certainly the result of inadvertant self-pollination of the interspecific F_1 plants that were used as seed parents in the back-cross. Emasculation was not performed, since these F_1's produced only about 7 seeds/100 manual self-pollinations. The coefficient of variability for the total population is 10.8%, but only 8.4%, if the two probable F_2 plants are excluded.

Selfed seed from plants of the above BC-F_1 progeny were grown out, and the frequency distribution of internal stigma lengths in the resulting BC-F_2 population appears in Fig. 5. The smooth and symmetrical distribution gives evidence neither of skewing nor of major gene segregation. The coefficients of variability of this population is 11.3%.

The F_1 hybrids produced by P. vulgaris 'Swiss' X P. coccineus 'Hammond's Dwarf White' crosses and by P. vulgaris 'Harvester' X P. coccineus PI 273666 crosses were backcrossed to their respective P. coccineus parents. In the backcross in which P. coccineus 'Hammond's Dwarf White' was used as seed parent, the progeny were distributed mostly between the parental means, with some overlap of the parental ranges (Fig. 9). Three of the 37 back-cross progeny had stigma types that fell within the 95% confidence region of 'Hammond's Dwarf White'. Overlap at the opposite end of the distribution could not be determined precisely, since only the internal stigma length mean of that parent was known. The distribution appears to be dis-continuous with a gap between extrorse and terminal types, although no such hiatus appears to separate terminal and introrse classes. The coef-ficient of variability for internal stigma length is 23.2%, which is larger than that of the F_2 population.

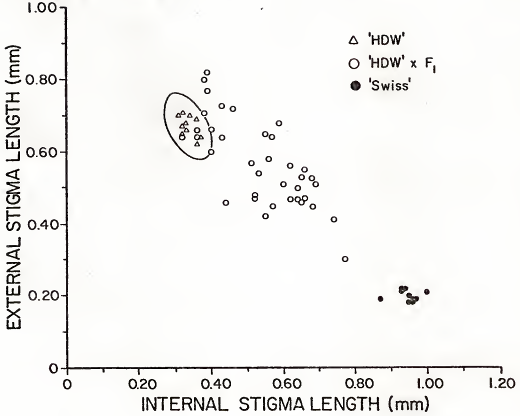


Figure 9. Distribution of stigma types in Phaseolus vulgaris 'Swiss', Phaseolus coccineus 'Hammond's Dwarf White' ('HDW'), and the backcross of the F_1 hybrid to 'Hammond's Dwarf White'. The ellipse represents a 95% confidence region for 'Hammond's Dwarf White'.

The 2-gene, dominant epistasis model predicts that the above test-cross should yield stigma types in the following ratio: 2 introrse (A__) : 1 terminal (aa_) : 1 extrorse (aabb). However, an obvious shortage of introrse types was noted in the backcross to 'Hammond's Dwarf White'. It was initially hypothesized that the shortfall was due to selection against zygotes with stigma alleles of P. vulgaris developing in P. coccineus cytoplasm. However, a similar distribution with a shortage of introrse stigma types was noted in the backcross to P. coccineus PI 273666, in which the F_1, with cytoplasm of P. vulgaris, served as seed parent (Fig. 10). The latter progeny is distributed continuously between the 95% confidence regions of the parents, and the coefficient of variability for internal stigma length is 29.7%. The reciprocal backcross employing P. coccineus PI 273666 as the female parent was also attempted, but this cross was difficult to achieve, and only 4 plants were produced after a difficult germination period (Fig. 10). The population was too small to be interpreted properly, but the stigma types were in the same range as that described above for the backcross utilizing the F_1 as seed parent.

Figure 11 is a series of histograms showing internal stigma length distributions, including progeny means and standard deviations, for 10 F_3 families derived from the cross P. vulgaris 'Swiss' X P. coccineus 'Hammond's Dwarf White'. It demonstrates that individuals drawn from different points in the F_2 distribution produce F_3 progenies with means that vary greatly within the limits of the parental means. The variability of the F_3 progenies also differs from family to family, being no less variable than the parental populations and no more variable than the F_2 population. Coefficients of variability range from 5.1% to 12.5%. These are characteristic features of a quantitative mode of inheritance.

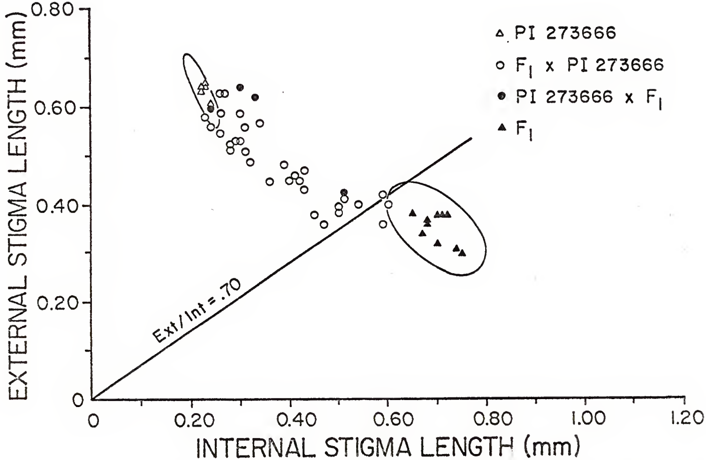


Figure 10. Distribution of stigma types in reciprocal backcrosses of the _Phaseolus vulgaris_ 'Harvester' X _Phaseolus coccineus_ PI 273666 F_1 hybrid to the _P. coccineus_ parent. Ellipses represent 95% confidence regions for populations upon which they are centered.

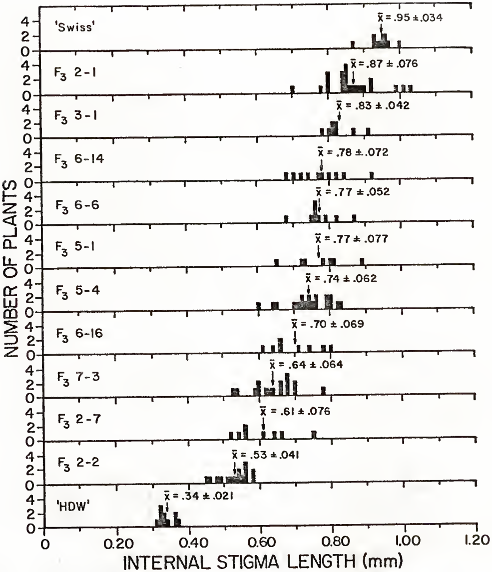


Figure 11. A comparison of internal stigma length distributions, with means and standard deviations, in 10 F_3 families derived from the crossing of Phaseolus vulgaris 'Swiss' X Phaseolus coccineus 'Hammonds's Dwarf White' ('HDW').

The internal stigma length means of the F_3 progenies above were strongly related to the internal stigma lengths of their respective F_2 parents. This fact has been illustrated in Fig. 12 by regressing F_3 means upon F_2 parents. The high correlation coefficient, r = 0.933, stresses the relatedness of the F_3 means and the F_2 parental values. An estimate of narrow sense heritability for internal stigma length was obtained by multiplying the correlation coefficient by the proper fraction for the inbred generations under consideration (Frey and Horner, 1957; Smith and Kinman, 1965). This procedure yielded a value of 62%. A similar regression of the Ext/Int stigma ratio of F_3 progeny means upon the Ext/Int ratio of F_2 parents is illustrated in Fig. 13 for materials derived from P. vulgaris 'Harvester' X P. coccineus PI line crosses. Again, the high correlation coefficient, r = 0.868, is indicative of the strong similarity between stigma types of F_2 plants and stigma types in their progenies. The narrow sense heritability estimate derived from the P. vulgaris 'Harvester' X P. coccineus PI line crosses is 58%.

Effect of Inbreeding on Stigma Inheritance and Fertility in Hybrid Materials

The 2-gene, dominant epistasis model of stigma inheritance describes three stigma classes controlled by the segregation of major genes. Two of these classes--the extrorse and terminal--were thought to be discreet types, while the third--the introrse type--was variable due to the influence of modifying factors. Difficulty in defining the boundary between the terminal class and the extreme of the introrse range with short internal stigma lengths led to problems in determining the frequencies of these classes in populations segregating for stigma type. In order to identify the boundary precisely, a range of F_2 plants with stigma types

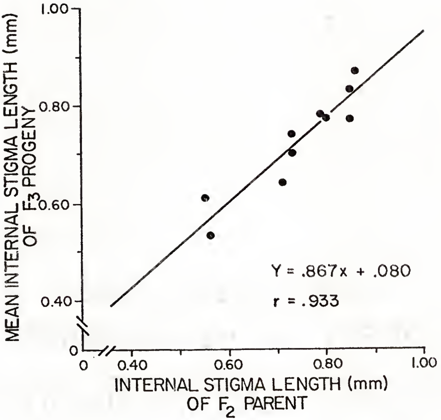


Figure 12. Regression of mean internal stigma lengths of 10 F_3 progenies ($n \geq 5$), derived from the crossing of Phaseolus vulgaris 'Swiss' X Phaseolus coccineus 'Hammond's Dwarf White', upon the internal stigma lengths of the F_2 parents.

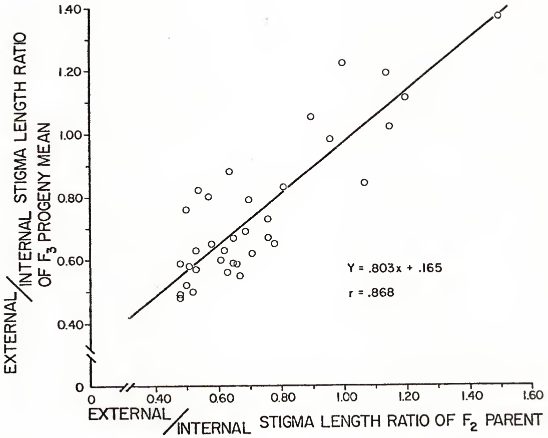


Figure 13. Regression of external/internal stigma length ratios of F_3 progeny means ($n \geq 5$), derived from the crossing of <u>Phaseolus vulgaris</u> 'Harvester' X <u>Phaseolus coccineus</u> PI lines, upon ratios of F_2 parents.

classified tentatively as introrse or terminal were inbred for a number of generations so that heterozygous genotypes of intermediate expression would be eliminated. It was hoped that as plants homozygous for stigma genes appeared in F_4 and F_5 generations, similar genotypes would tend to form clusters when plotted in scatter diagrams. This would permit the boundary between the terminal and introrse classes to be visualized and perhaps provide some information about the number of modifying factors influencing the introrse class.

F_2 plants derived from P. vulgaris 'Harvester' X P. coccineus PI line crosses were inbred to the F_4, and in some cases, to the F_5 generation. The approach to homozygosity within progenies was monitored by computing determinants of progeny covariance matrices ($|S|$), which indicate the degree of dispersion of points in 2-dimensional scatter diagrams. By this measure, the P. vulgaris cultivar 'Harvester' has an $|S|$ value of 2×10^{-7}. For the purposes of the inbreeding experiment, F_3, F_4 and F_5 progenies with $|S| < 2 \times 10^{-6}$ were considered essentially homozygous for stigma genes. Twenty-six such progenies were identified, and these have been plotted in a 2-dimensional scatter diagram (Fig. 14). The diagram shows that far from forming discreet groupings of points, the means of progenies near homozygosity are continuously distributed in the transition range between the proposed terminal and introrse "classes." This seems to indicate that modifiers influence stigma shape in the terminal class as well as in the introrse class. On the basis of this result, the model hypothesizing major gene control of stigma segregation into definable classes seems questionable.

During the inbreeding experiment, it was necessary to emphasize selection for stigma types on the extrorse end of the distribution in order to

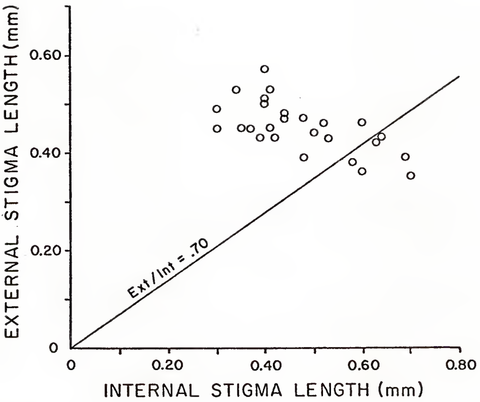


Figure 14. Distribution of mean stigma lengths of 26 F_3, F_4 and F_5 progenies ($n \geq 4$) derived from crosses of P. vulgaris 'Harvester' X P. coccineus PI lines. The determinant of the progeny covariance matrix, $|S|$, an estimator of stigma variability within progenies, is less than 2.0×10^{-6} for each of the above progenies, indicating a condition approaching homozygosity for stigma genes.

extend the range of the investigation to include stigmas with Ext/Int ratios greater than 1.0. Selection was successful in moving the means of successive generations toward the extrorse type (Table 9). Following the initial selection of F_2 parents, progress toward higher Ext/Int ratios was primarily the result of decreases in internal stigma length, rather than increases in external stigma length, in spite of the fact that selection was based on the Ext/Int ratio, rather than solely on internal stigma length. This seems to indicate the possibility of some independence in the action of "internalizing" and "externalizing" genes, in that those governing external extent can become fixed before those controlling internal extent.

A great deal of stigma variability was displayed in the progeny of some F_2 plants. Figure 15 shows progeny descended from 2 F_2 plants, F_2 66-7 and F_2 66-1, which had Ext/Int ratios near the mean of the general F_2 population (Ext/Int = 0.46). During inbreeding, selection for large Ext/Int ratios resulted in some F_4 and F_5 progenies with mean Ext/Int ratios greater than 1.60 and several others with intermediate ratios. The downward trend of Fig. 15 in moving from F_2 to F_5 progenies indicates the increase in homozygosity with inbreeding. The upward jog from the F_3 mean of F_2 66-7 to the selected F_4 mean is anomalous and can only be attributed to an inaccurate estimate of stigma shape variability in these two progenies due to sampling error.

The approach to homozygosity with inbreeding is indicated in Table 9, which gives the pooled progeny $|S|$ values for each generation from F_2 to F_5. Confidence limits computed for the pooled $|S|$ values do not overlap, indicating that stigma variability within progenies decreased significantly with each generation of inbreeding from F_2 to F_5.

Table 9. Response to selection for terminal and extrorse stigma types during inbreeding of materials derived from hybridizations of <u>Phaseolus vulgaris</u> 'Harvester' X <u>Phaseolus coccineus</u> PI lines. Shifts in mean internal stigma length ($\overline{Int}$), in mean external stigma length ($\overline{Ext}$) and in stigma shape variability within progenies ($|S| \times 10^6$) during inbreeding from F_2 to F_5 are listed, along with 95% confidence limits for $|S| \times 10^6$.

Generation	Number of Progenies	Number of Plants	$\overline{Int}$	$\overline{Ext}$	$\overline{Ext}/\overline{Int}$	Pooled $\lvert S\rvert \times 10^6$	95% C.L. for $\lvert S\rvert \times 10^6$ Lower	95% C.L. for $\lvert S\rvert \times 10^6$ Upper
F_2	4	203	0.76	0.35	0.46	49.88	39.03	69.07
F_3	37	376	0.57	0.42	0.74	10.93	9.01	13.89
F_4	36	220	0.52	0.45	0.87	5.49	4.26	7.72
F_5	7	57	0.38	0.44	1.16	0.80	0.51	1.80

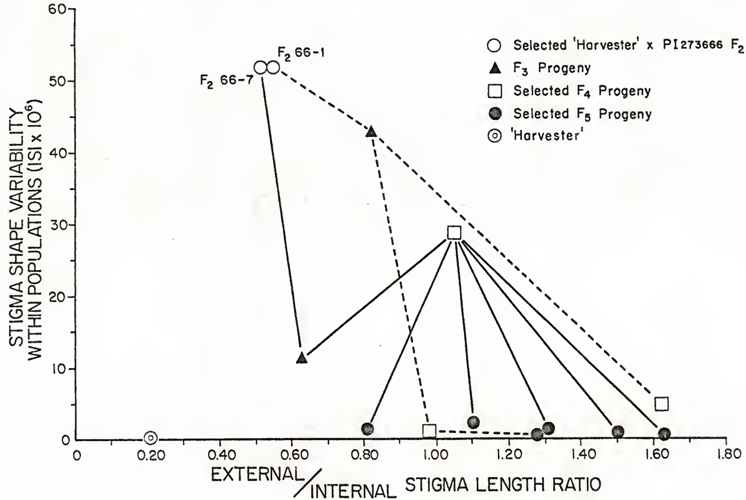


Figure 15. Response to selection for terminal and extrorse stigma types in inbred generations derived from 2 F_2 progeny (F_2 66-1 and F_2 66-7) of the cross _Phaseolus vulgaris_ 'Harvester' X _Phaseolus coccineus_ PI 273666. Stigma length ratios in F_3, F_4 and F_5 generations are for progeny means. The variability of stigma types indicated for F_2 66-1 and F_2 66-7 refers to the entire F_2 population from which they were selected.

In spite of the fact that selection of inbred materials was made on the basis of fertility as well as stigma type, the mean fertility level of inbred plants, determined by the fertility index described previously, was observed to decline with each generation (Table 10). The large standard deviations indicate that fertilities varied widely within generations, and fertility differences between generations could not be shown to be statistically significant. In most cases, sterility was so complete that it was impossible to continue the inbreeding experiment beyond the F_4 generation due to a failure to produce sufficient F_5 seed. A normal common bean plant that sets 15 pods with 6 seeds per pod would have a fertility index of 540. None of the materials in this study approached normal fertility, although several F_2 plants did have an appreciable capacity for self-pollination. Self-pollinated seeds volunteered by F_3, F_4 and F_5 plants were very rare. This may have been due, in part, to a shift in mean stigma shape toward the extrorse type of the outcrossing _P. coccineus_ parent, but sterility probably played a larger role. No marked differences in mean fertility were observed for plants with Ext/Int ratios less than 0.70, as opposed to those with ratios greater than 0.70. This seems to suggest that factors affecting fertility are independent of stigma genes. However, the fertility aspects of this study were secondary objectives, and the data obtained are not sufficiently precise to conclusively establish independence.

Recovery of Selected Stigma Types in Segregating Populations Produced by Backcrossing to _P. vulgaris_

The terminal and extrorse segregates (F_2 1-2, F_2 2-7, F_2 2-8, F_2 6-7 and F_2 8-2) from the F_2 population produced by crossing _P. vulgaris_ 'Swiss' X _P. coccineus_ 'Hammond's Dwarf White', and also the terminal segregants

Table 10. Depression of fertility with inbreeding in materials derived from hybridization of _Phaseolus vulgaris_ 'Harvester' X _Phaseolus coccineus_ PI lines. Calculations of the mean fertility index (see Methods and Materials, p. 54) and mean number of seeds volunteered per plant distinguish within generations between plants with terminal or extrorse stigmas (Ext/Int ≥ 0.70) and those with introrse stigmas (Ext/Int < 0.70).

	Number of Plants	Mean Fertility Index	Average Number of Volunteered Seeds / Plant
F_2			
Ext/Int < 0.70	55	10.1 ± 28.1	11.0 ± 20.0
Ext/Int ≥ 0.70	11	11.6 ± 12.2	2.1 ± 6.6
F_3			
Ext/Int < 0.70	120	4.1 ± 6.5	0.6 ± 2.7
Ext/Int ≥ 0.70	126	3.5 ± 8.1	0.2 ± 0.8
F_4			
Ext/Int < 0.70	48	1.9 ± 3.2	0.4 ± 1.2
Ext/Int ≥ 0.70	104	2.2 ± 7.3	0.1 ± 0.4

(BC 2-15 and BC 4-22) from the population produced by backcrossing the 'Swiss' X 'Hammond's Dwarf White' F_1 to P. vulgaris, were backcrossed to P. vulgaris 'Light Red Kidney' and to the P. vulgaris breeding line F_3-1. The backcrosses to P. vulgaris were undertaken with the intent of recovering terminal and extrorse stigma types in P. vulgaris-like phenotypes with improved fertility. Large, field-grown BC-F_2 populations, consisting of up to 976 individuals, were produced to facilitate recovery of the desired stigma types.

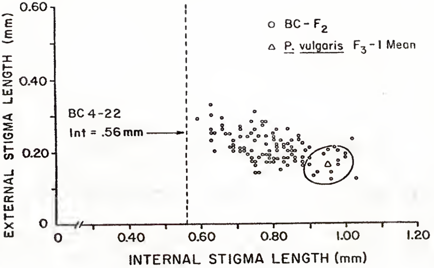
The backcross of the terminal segregant BC 4-22 to P. vulgaris breeding line F_3-1 resulted in the BC-F_2population illustrated in Fig. 16. The distribution of stigma types appears to be continuous in the range between the parents. Whereas 14 BC-F_2 individuals out of the total BC-F_2 population of 105 plants fell within the P. vulgaris confidence region, no segregants were recovered having internal stigma lengths equal to, or less than, the BC 4-22 parent.

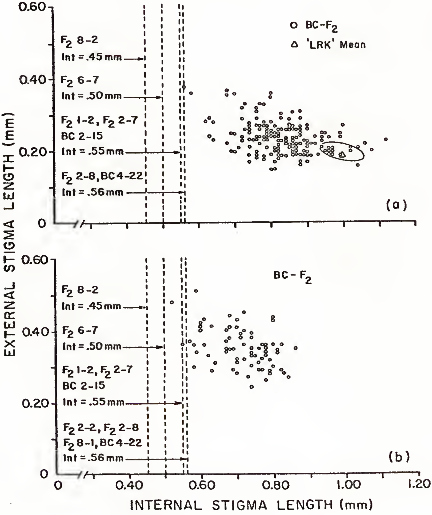
A similar result was obtained in the BC-F_2 population produced by backcrossing 7 terminal and extrorse segregants, derived from crossing P. vulgaris 'Swiss' X P. coccineus 'Hammond's Dwarf White', to P. vulgaris 'Light Red Kidney', as illustrated in Fig. 17(a). The population formed by combining the 7 BC-F_2 populations resembles that in Fig. 16. The introrse tail of the distribution overlaps the 'Light Red Kidney' confidence region, while only 2 or 3 segregants approach or equal the parental types at the extrorse end of the distribution.

Figure 17(b) presents the distribution of stigma types in the extrorse tail of a large, field-grown BC-F_2 population containing 976 plants produced by combining the BC-F_2 progenies of 9 terminal and extrorse segregants backcrossed to P. vulgaris 'Light Red Kidney'. Thus, the

Figure 16. Distribution of stigma types in a BC-F_2 population produced by backcrossing a _P. vulgaris_ 'Swiss' X _P. coccineus_ 'HDW' segregant (BC 4-22) having short internal stigma length (vertical dashed line) to _P. vulgaris_ breeding line F_3-1. Ellipse is 95% confidence region for _P. vulgaris_ breeding line F_3-1.

Figure 17. Distribution of stigma types in BC-F_2 populations produced by backcrossing _P. vulgaris_ 'Swiss' X _P. coccineus_ 'HDW' segregants having short internal stigma lengths (vertical dashed lines) to _P. vulgaris_ 'Light Red Kidney' (LRK). (a) BC-F_2 consisting of the combined progenies of 7 'Swiss' X 'HDW' F_2 plants backcrossed to _P. vulgaris_ 'LRK'. Ellipse is 95% confidence region for _P. vulgaris_ 'LRK'. (b) The extrorse extreme of stigma type distribution in a large, field-grown BC-F_2 population (n = 976) consisting of the combined progenies of 9 'Swiss' X 'HDW' F_2 plants backcrossed to _P. vulgaris_ 'LRK'.


0.60
0.40
0.20
0
EXTERNAL STIGMA LENGTH (mm)
o BC - F2
Δ P. vulgaris F3 - I Mean
BC 4-22
Int = .56 mm
0.40
0.60
0.80
1.00
1.20
INTERNAL STIGMA LENGTH (mm)


0.60
0.40
0.20
0
o BC-F2
Δ 'LRK' Mean
F2 8-2
Int = .45 mm
F2 6-7
Int = .50 mm
F2 1-2, F2 2-7
BC 2-15
Int = .55 mm
F2 2-8, BC 4-22
Int = .56 mm
(a)
EXTERNAL STIGMA LENGTH (mm)
BC - F2
F2 8-2
Int = .45 mm
F2 6-7
Int = .50 mm
F2 1-2, F2 2-7
BC 2-15
Int = .55 mm
F2 2-2, F2 2-8
F2 8-1, BC 4-22
Int = .56 mm
(b)
0.40
0.60
0.80
1.00
1.20
INTERNAL STIGMA LENGTH (mm)

pedigrees in the population in Fig. 17(b) were very similar to those in the population illustrated in Fig. 17(a). The increased population size defined more clearly the extrorse tail of the BC-F_2 population, but again very few segregants had internal stigma lengths as short, or shorter, than those of the terminal parents.

The 2-gene, dominant epistasis model of stigma inheritance predicts that terminal and extrorse stigma types should be recovered in one quarter of the BC-F_2 progeny. The difficulty experienced in obtaining any such segregants, even in large populations, suggested that the a allele contributed by P. coccineus was not being transmitted with the expected frequency in the backcross to P. vulgaris. It was hypothesized that some selective elimination of the a stigma allele was occurring in the backcrossing procedure.

Also included in the backcrossing program were plants with Ext/Int stigma ratios greater than 0.70 (terminal and extrorse types) from the F_2, F_3 and F_4 generations derived from hybridization of P. vulgaris 'Harvester' and P. coccineus PI lines. Toward the same goal of recovering the terminal and extrorse stigma types in fertile, P. vulgaris phenotypes, the above materials were backcrossed to the following P. vulgaris cultivars: 'Harvester', 'Sprite', 'Light Red Kidney' and the breeding line 6-19. The BC-F_2 populations were grown in large,field plots with up to 4000 individuals per population.

Figure 18(a) shows the BC-F_2 population produced by backcrossing several F_2 and F_3 plants to P. vulgaris 'Harvester'. Although the rate of recovery of plants with Ext/Int stigma ratios greater than 0.70 is fairly large (4 out of 97) in this subsample from the population shown in Fig. 18(b), the shape and extent of the distribution are thought to be

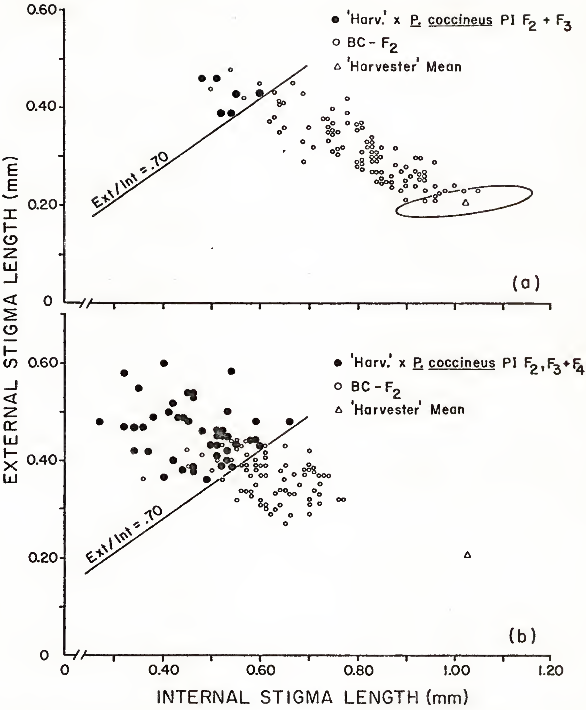


Figure 18. Distribution of stigma types in BC-F_2 populations produced by backcrossing F_2, F_3 and F_4 plants (derived from <u>Phaseolus vulgaris</u> 'Harvester' X <u>Phaseolus coccineus</u> PI line crosses) having Ext/Int stigma ratios greater than 0.70 to 'Harvester'. (a) Representative BC-F_2 population consisting of the combined progenies of 6 F_2 and F_3 plants backcrossed to <u>P. vulgaris</u> 'Harvester'. The ellipse is a 95% confidence region for 'Harvester'. (b) The extrorse extreme of stigma type distribution in a large, field-grown BC-F_2 population (n = 1641) consisting of the combined progenies of 45 'Harv.' X PI F_2, F_3 and F_4 plants backcrossed to 'Harvester'.

typical of BC-F_2 populations. Figure 18(b) shows the extrorse tail of the distribution of stigma types in a large, field-grown BC-F_2 population. The low recovery rate of terminal and external stigma types (15 out of 1641) is very similar to the result illustrated in Fig. 17(b).

In other BC-F_2 populations of this series, recovery rates for plants with Ext/Int ratios greater than 0.70 ranged from 0.8% to 1.8% of the total population (Table 11). This is in marked contrast to the frequency of such stigmas in the F_2 of the original crosses between P. vulgaris 'Harvester' and P. coccineus PI lines, where 15.3% of the plants had Ext/Int stigma ratios greater than 0.70.

Certain BC-F_2 lines derived from particular BC-F_1 plants transmitted the a allele with greater than average frequency, in some cases equal to the transmission rate in the original interspecific F_2 population. This was true of BC-F_2 lines 1108, 1114, 1133 and 1134 produced by backcrossing 2 terminal F_2 plants to 'Sprite' (Table 11). It was assumed that in these lines a linkage had been broken between the a stigma allele and some recessive, deleterious gene that, in the homozygous condition, reduced the recovery rate of the desired stigma types below the 25% rate expected according to the 2-gene, dominant epistasis model. Selections from these lines were inbred for one generation, and the most fertile BC-F_3 plants with stigma ratios greater than 0.70 were again backcrossed to 'Sprite'. With the breaking of the hypothetical linkage, it was expected that high rates of transmission of the a allele would continue into the BC_2-F_2 generation with improved fertility. However, as Table 11 shows, the recovery rate of plants with Ext/Int ratios greater than 0.70 was actually slightly less than that observed in the first round of backcrossing. In another large BC_2-F_2 population utilizing the breeding line 7-1404 as the

Table 11. Rate of recovery of terminal and extrorse segregants (Ext/Int ≥ 0.70) in F_2 and BC-F_2 populations derived from crosses of *Phaseolus vulgaris* 'Harvester' X *Phaseolus coccineus* PI lines. The BC-F_2 populations were produced by backcrossing selected F_2, F_3 and F_4 plants having terminal or extrorse stigmas with various *P. vulgaris* cultivars and breeding lines.

Population	Number of Parents with Ext/Int ≥ 0.70	Population n	Number of Recovered Plants with Ext/Int ≥ 0.70	% Recovery
'Harv.' X PI F_2	4	203	31	15.3
'LRK' Backcross				
BC_1-F_2	2	263	2	0.8
6-19 Backcross				
BC_1-F_2	1	718	9	1.3
'Harv.' Backcrosses				
BC_1-F_2	45	1641	15	0.9
BC_2-F_2 (7-1404 ♀)	5	2722	2	0.1
'Sprite' Backcrosses				
BC_1-F_2	8	1762	32	1.8
1108	1	30	2	6.7
1114	1	23	3	13.0
1133	1	33	5	15.2
1134	1	29	5	17.2
BC_2-F_2	17	4136	48	1.2

recurrent *P. vulgaris* parent, terminal stigmas comprised only 0.1% of the population. In the production of this BC_2-F_2 population, no inbreeding preceded the second backcross.

One of the reasons for pursuing the backcrosses to *P. vulgaris* was to improve the fertility of terminal and extrorse selections. While the quality of the fertility data are again questionable, there does not seem to be any dramatic improvement in fertility from the BC_1-F_2 to the BC_2-F_2 generation (Table 12). For that matter, a comparison with Table 10 shows that overall fertility in the BC-F_2 generations was not improved beyond the fertility level of the interspecific F_2 generation. Contrary to the results in Table 10, Table 12 suggests that plants with Ext/Int stigma ratios greater than 0.70 may tend to be more sterile than the more introrse group.

Test of Selective Elimination of Stigma Alleles in Reciprocal Testcrosses

Several different observations made during the course of this study have indicated that selective elimination of stigma alleles occurs. Backcrosses of interspecific F_1 plants to the *P. coccineus* parent have revealed in two different populations that plants carrying the *A* stigma allele of *P. vulgaris* were fewer than expected. In the F_2 populations resulting from the hybridization of *P. vulgaris* 'Harvester' and *P. coccineus* PI lines, a shortage of stigma types determined by the *a* stigma allele of *P. coccineus* was noted. Finally, in the large, field-grown BC-F_2 populations segregating for stigma types, the recovery of plants homozygous for *a* was drastically less than expected. All of these discrepancies are with reference to expectations predicted by the 2-gene, dominant epistasis model of stigma inheritance described earlier in this section.

Table 12. Fertility of plants with terminal or extrorse stigmas (Ext/Int $\geq$ 0.70) as opposed to those with introrse stigmas (Ext/Int < 0.70) after 1 (BC_1-F_2) or 2 (BC_2-F_2) rounds of backcrossing to P. vulgaris.

	Number of Plants	Mean Fertility Index	Average Number of Volunteered Seeds/Plant
'Harvester' Backcrosses			
BC_1-F_2			
Ext/Int < 0.70	17	14.5 ± 30.5	--
Ext/Int ≥ 0.70	15	11.5 ± 14.4	--
BC_2-F_2 (7-1404 ♀)			
Ext/Int < 0.70	41	17.6 ± 34.0	25.7 ± 29.7
Ext/Int ≥ 0.70	2	6.3 ± 8.8	35.0 ± 35.4
'Sprite' Backcrosses			
BC_1-F_2			
Ext/Int < 0.70	5	20.9 ± 19.8	--
Ext/Int ≥ 0.70	13	0.1 ± 0.3	--
BC_2-F_2			
Ext/Int < 0.70	23	12.0 ± 19.0	12.2 ± 19.5
Ext/Int ≥ 0.70	48	5.1 ± 8.3	5.2 ± 8.6

One mechanism of selective elimination, pollen competition, was examined in this study to determine whether it might be the cause of the observed deviations from expected segregation ratios. It was hypothesized that the shortage of genotypes homozygous for the a allele in interspecific F_2 and BC-F_2 populations could be the result of a selective disadvantage suffered by pollen grains carrying the a allele of P. coccineus when competing for unfertilized ovules with pollen grains carrying the A allele of P. vulgaris. In order to test this hypothesis, it was proposed that reciprocal testcrosses be made between lines homozygous for the a allele (aa), on the one hand, and lines heterozygous for stigma alleles (Aa), on the other. If the stigma alleles or closely linked genes were the cause of competitive effects, the progenies resulting from a reciprocal cross should differ with respect to stigma type composition. Pollination of aa plants with Aa pollen whould result in selective elimination of a pollen due to unfavorable competition with A pollen. The resulting progeny should consist largely of Aa plants with a uniform stigma type resembling that of the heterozygous parent. The reciprocal cross, that of aa pollen on Aa plants, should result in equal numbers of individuals in two discrete groups corresponding to the parental stigma types.

The homozygous (aa) sources employed in this experiment were F_5 inbred progenies, F_5 66-7-5-3 and F_5 66-1-1-4, derived from hybridization of P. vulgaris 'Harvester' and P. coccineus PI 273666. These progenies were quite uniform for stigma type, having $|S|$ values less than 5×10^{-7}. It was assumed that both were approximately homozygous for genes affecting stigma shape. The heterozygous (Aa) parents for the reciprocal crosses were produced by backcrossing the F_4 parents of the F_5 progenies mentioned above to P. vulgaris 'Harvester' ($|S| = 2 \times 10^{-7}$). These will be referred

to simply as BC-F_1 plants. In making the reciprocal crosses in the greenhouse, it was discovered that one of the F_5 lines, F_5 66-1-1-4, would set no seed even after repeated cross-pollinations. Using the F_5 as a pollen parent, crosses onto the related BC-F_1 progeny were successful. Reciprocal crosses involving F_5 66-7-5-3 were successful in both crossing directions. The BC-F_1 plants, being vigorous and fertile, produced more seed than the inbred F_5 progeny.

The reciprocal progenies resulting from the crosses involving F_5 66-7-5-3 were tested to determine whether differences in stigma type distribution existed. Differences in mean internal stigma length, variances of internal and of external stigma lengths, and regression coefficients were found not to be significant (Table 13). A significant difference in mean external stigma length amounted to only 4.7% of the smaller mean value. A chi-square test of independence showed that the direction of crossing had no significant effect upon the distribution of internal stigma lengths in the reciprocal progenies (Table 14). On the basis of these tests, it was concluded that no reciprocal differences in the distributions of stigma types existed in the reciprocally crossed progenies. Thus, it can be concluded that pollen competition is not a factor in these crosses, and the _a_ stigma allele of _P. coccineus_ confers no selective disadvantage upon pollen grains carrying it. The discrepancies between observed and expected stigma segregations in F_2 and BC-F_2 populations, mentioned previously, must have a different cause.

In order to eliminate the possibility that the materials used in the testcrosses constitute a special case, it was necessary to show that the BC-F_2 populations derived from them did not contain a higher frequency of terminal and extrorse stigma types than had been previously noted in

Table 13. Tests for significant differences in internal stigma length means and variances, external stigma length means and variances, and regression coefficients of 2 populations produced by reciprocally crossing a BC-F_1 line ('Harvester' X PI 273666 inbred to F_4 and backcrossed to 'Harvester' as female) with an F_5 line derived by selfing the above F_4.

	Number of Plants	Internal Stigma Length		External Stigma Length		Regression Coefficient
		Mean (mm)	Variance	Mean (mm)	Variance	
BC-F_1 X F_5	132	0.58	0.01254	0.40	0.00270	- 0.340
F_5 X BC-F_1	60	0.57	0.01188	0.39	0.00260	- 0.406
t		n.s.	--	*	--	n.s.
F		--	n.s.	--	n.s.	--

* Significant at 5% level.

Table 14. Chi-square test of independence for direction of crossing and internal stigma length distribution in 2 populations produced by reciprocally crossing a BC-F_1 line ('Harvester' X PI 273666 inbred to F_4 and backcrossed to 'Harvester' as female) with an F_5 line derived by selfing the above F_4.

	Number of Plants								
		Internal Stigma Length (mm)-Upper Class Limits							
	Total	0.40	0.50	0.60	0.70	0.80	0.90	X^2	P
BC-F_1 X F_5	132	5	35	38	36	16	2		
								0.697	0.99-0.975
F_5 X BC-F_1	60	2	18	15	18	6	1		

other BC-F_2 populations. A total of 388 seeds that volunteered on BC-F_1 plants in protected, winter greenhouses were planted in the field to form the BC-F_2 generation. In the populations derived from F_4 66-7-5-3 and F_4 66-1-1-4, the frequency of plants with Ext/Int stigma ratios greater than 0.70 was 0.7% and 0.4%, respectively. These figures correspond well to the results observed previously for other BC-F_2 populations.

As the seed for the reciprocal cross experiment was harvested, notes were taken on the number of mature seeds, the number of partially developed and subsequently aborted seed, and on the number of ovules which for one reason or another did not develop at all (Table 15). This was done to determine whether ovule or zygote abortion might be involved in reciprocal differences in transmission of stigma alleles. As the data in Table 15 show, the number of unfertilized ovules (minimims) is nearly uniform in all three crosses. The data for seed production and abortion on both BC-F_1 progenies are also similar. However, the F_5 plants showed more embryo abortion and less seed production than BC-F_1 plants. In light of the fact that there was no reciprocal difference in distribution of stigma types in populations produced from F_5 plants, as opposed to BC-F_1 plants, it is assumed that the higher abortion rate on the F_5 plants reflects a less vigorous maternal environment incapable of supporting more than about 1.5 seeds/pod, rather than selective elimination of zygotes or ovules carrying deleterious stigma alleles. The 2 BC-F_1 progenies yielded averages of 2.9 seeds/pod and 2.3 seeds/pod.

The distribution of stigma types in the progenies of the reciprocal crosses, which have been combined in Fig. 19, and in the unidirectional cross involving the F_5 66-1-1-4 family, which is illustrated in Fig. 20, provides insight into the genetic control of stigma shape. The distribution is quite different from either of the alternatives predicted by

Table 15. A comparison of ovule abortion in reciprocally crossed progenies derived from the _P. vulgaris_ 'Harvester' X _P. coccineus_ PI 273666 F_4's 66-1-1-4 and 66-7-5-3.

	Total Number of Ovules	Percent of Ovules		
		Undeveloped Ovules	Aborted Ovules	Mature Seeds
F_4 66-1-1-4				
$BC-F_1 \times F_5$	192	27.6	8.3	64.1
F_4 66-7-5-3				
$BC-F_1 \times F_5$	296	36.1	11.8	52.0
$F_5 \times BC-F_1$	69	30.4	31.5	38.1

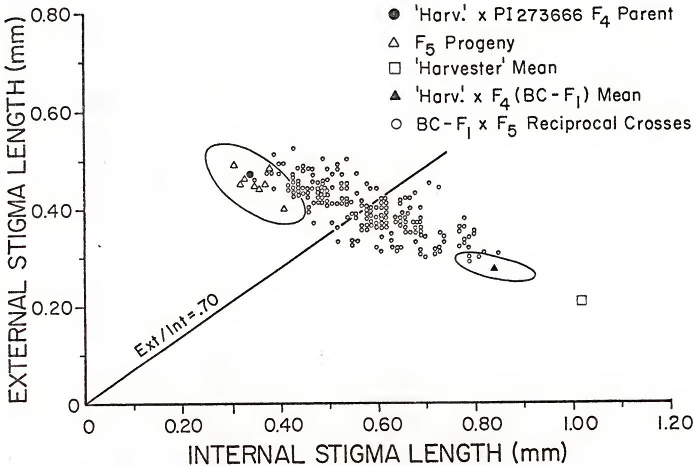


Figure 19. Distribution of stigma types in a population composed of two combined progenies produced by reciprocally crossing a BC-F_1 line ('Harvester' X PI 273666 F_2 66-7 inbred to F_4 and backcrossed to 'Harvester' as female) with an F_5 line derived by selfing the above F_4.

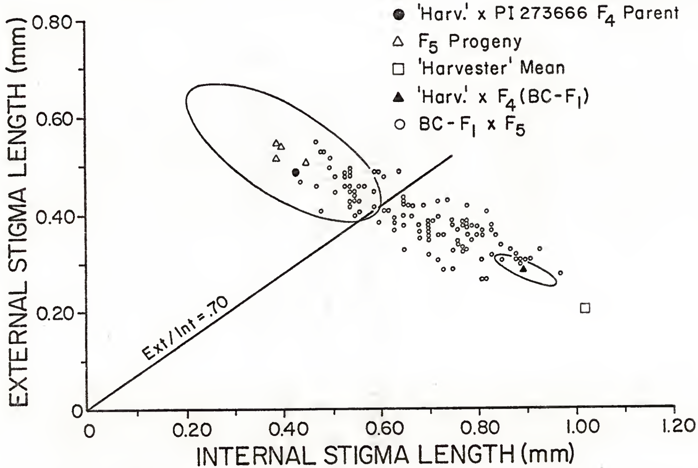


Figure 20. Distribution of stigma types in a population produced by crossing a BC-F_1 line ('Harvester' X PI 273666 F_2 66-1 inbred to F_4 and backcrossed to 'Harvester' as female) with an F_5 line derived by selfing the above F_4.

the 2-gene, dominant epistasis model. Rather than being discontinuous and centered on the parental types, the progenies are continuously and symmetrically distributed between the parental means. In short, instead of a simple Mendelian segregation pattern, stigma shape clearly displays a quantitative mode of inheritance in these interspecific materials. Judging from the number of individuals in the segregating population that fall within the confidence regions about the F_5 progenies, a minimum of 2 to 4 genes control segregation in these materials. This is undoubtedly a conservative estimate, since the confidence regions about the F_5 progenies are inflated by small sample sizes and some minor variability in stigma types within F_5 progenies. A better estimate based on the smaller confidence regions about the highly uniform BC-F_1 progenies indicates that a minimum of 5 to 7 genes are involved in control of stigma shape over the range between the F_5 progeny and the 'Harvester' mean.

The failure of the 2-gene, dominant epistasis model to accurately predict segregation in the reciprocal cross progenies makes all of the hypotheses based upon it very doubtful. Among these are the contention that discrete introrse, terminal, and extrorse stigma classes exist; the proposal that the _A_ allele of _P. vulgaris_ is selectively eliminated in the backcross of F_1 hybrids to _P. coccineus_; and the claim of selective elimination of the _a_ allele of _P. coccineus_ in backcrosses to _P. vulgaris_. Evidence of the quantitative and polygenic nature of stigma shape inheritance permits a different and more satisfactory interpretation of the experimental results.

CONCLUSIONS

The 2-gene, dominant epistasis model of stigma inheritance was proposed primarily on the basis of segregation observed in a single interspecific F_2 population early in the course of this study. This model subsequently failed to explain segregation in other F_2 populations, in backcrosses of the F_1 hybrids to P. coccineus, in large, field-grown BC-F_2 populations produced by backcrossing inbred materials with terminal and extrorse stigmas to P. vulgaris, and in the testcrosses just described in the previous section. Selective elimination of the a stigma allele, a hypothesis proposed to explain the discrepancy between the segregation ratios observed in BC-F_2 populations and those expected on the basis of the 2-gene model, was shown not to occur in the reciprocal testcross experiment. This left the model bankrupt as an explanation of stigma inheritance.

The data conform much more satisfactorily to a quantitative model of inheritance. East (1916) outlined the segregation patterns characteristic of quantitative traits when all populations succeeding the original hybridization are obtained by self-fertilization. These serve as a useful guide to the analysis of stigma inheritance in the early generations of the present study. Stigma variability in parental and F_1 populations is uniformly low and maximum variability is found in the F_2 generation (Figs. 5 and 6). Distributions of stigma types in segregating populations are continuous, and evidence to the contrary, as in the P. vulgaris 'Swiss' X P. coccineus 'Hammond's Dwarf White' F_2 and the backcross of the 'Swiss'

X 'Hammond's Dwarf White' F_1 to the *P. coccineus* parent (Fig. 9), are best attributed to small sample sizes. With the exception of the F_2 produced by crossing *P. vulgaris* 'Harvester' X *P. coccineus* PI 311819, F_2 populations were too small to recover parental stigma types (Figs. 7 and 8). Individuals from different points on the F_2 frequency curve produced F_3 families that differed considerably in their means, as well as their variability (Fig. 11). Variability in F_3, F_4 and F_5 families covered a range of values intermediate between those of the parents and the F_2 generation, the variability of any given family being generally less than that of the population from which it came (Fig. 15).

The evidence from the distribution of means of advanced inbred lines approaching homozygosity for stigma genes also favors a quantitative interpretation (Fig. 14). The continuous distribution of progeny means in the region, where the 2-gene model predicts a clear separation between terminal and introrse stigma classes, indicates that many genes are involved in stigma expression.

The relative infrequency with which terminal and extrorse stigma types were recovered in BC-F_2 populations is explained by the quantitative model in terms of the low probability of recovering, in an F_2 population, the full expression of a trait governed by many genes (Figs. 16, 17 and 18). If, for example, 6 genes segregate for stigma shape in forming the BC-F_2 population, only 1 plant in 4096 would be expected to recover the full extrorse genotype of the parent.

The results of the reciprocal testcross experiment indicate that a minimum of 5 to 7 genes are involved in the control of stigma shape over the range between the extrorse stigma parent, F_4 66-7-5-3, and *P. vulgaris* 'Harvester'. It can be concluded that even more genes must be involved in

segregation over the entire range of stigma types separating P. vulgaris and P. coccineus.

Use of the quantitatively inherited stigma trait in producing outcrossing common beans is hampered by the large population sizes and considerable field effort needed to obtain rare terminal or extrorse segregants. However, these can be found, as was demonstrated in the large $BC\text{-}F_2$ populations produced for this study. A much more serious problem is the high level of sterility that was evident in all of the $BC\text{-}F_2$ materials, but seemed to be most severe in those plants with Ext/Int stigma ratios greater than 0.70 (Table 12). The nature of this sterility, which remained formidable through 2 cycles of backcrossing to P. vulgaris, is not well understood. However, the possibility is not excluded that sterility factors may be linked to an important block of P. coccineus stigma alleles within an inverted chromosome segment of the type observed in similar materials by Cheng (1979). This arrangement would serve to protect the linkage and would account for the lack of improvement in fertility with backcrossing. Alternatively, there is nothing in the data that precludes the possibility of pleiotropism in the relationship between stigma type and sterility. Regardless of the cause of the observed sterility, it is noteworthy that selection for the more fertile terminal segregants in $BC_1\text{-}F_2$ and $BC_1\text{-}F_3$ populations did not result in improved fertility in the derived $BC_2\text{-}F_2$ populations.

Practically speaking, the sterility of the $BC\text{-}F_2$ progeny makes it difficult to properly evaluate the outcrossing potential of segregates with terminal stigma types. Semi-sterile plants which produce no seeds under cages in the field may become self-pollinating upon recovery of full fertility.

Supposing that it were possible to combine full fertility with a quantitatively inherited terminal or extrorse stigma type, use of such material in recurrent selection breeding programs would be restricted by the fact that only a small percentage of the population would segregate for the outcrossing stigma type after the initial hybridization with normal introrse stigma types. The quantitative mode of inheritance reduces the effectiveness of stigma type as a factor promoting outcrossing in segregating populations. It now seems unlikely that stigma alleles of *P. coccineus* will be employed in *P. vulgaris* germplasm to provide an outcrossing mechanism suitable for use in recurrent selection programs.

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BIOGRAPHICAL SKETCH

Richard Michael Manshardt was born in Rockford, Illinois, on September 14, 1945--the second son of Dr. Donald O. Manshardt, pathologist, and Mrs. Betty Schilling Manshardt, homemaker. He grew up and attended elementary school in Peoria, Illinois. Throughout his childhood, his chief abiding interest was in natural history. He graduated from Shattuck School in Faribault, Minnesota, in 1963, and attended Antioch College in Yellow Springs, Ohio, graduating with a B.A. in biology in 1969. He served in the U.S. Navy from 1969 to 1973, during which time he was stationed in Puerto Rico and the Far East. Upon his discharge from military service, he studied under Dr. Jack R. Harlan of the Crop Evolution Laboratory, University of Illinois. He received an M.S. in agronomy from the University of Illinois in 1976. An interest in legume crops led him to undertake a doctoral program in common bean genetics and breeding with Dr. Mark J. Bassett at the University of Florida.

He is married to Elizabeth Anne Allingham.

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

Mark J. Bassett

Mark J. Bassett, Chairman
Associate Professor of Horticultural Science

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

Charles E. Dean

Charles E. Dean
Professor of Agronomy

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

Larkin C. Hannah

Larkin C. Hannah
Associate Professor of Horticultural Science

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

Donald N. Maynard

Donald N. Maynard
Professor of Horticultural Science

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

Wayne B. Sherman

Wayne B. Sherman
Professor of Horticultural Science

This dissertation was submitted to the Graduate Faculty of the College of Agriculture and to the Graduate Council, and was accepted as partial fulfillment of the requirements for the degree of Doctor of Philosophy.

December 1980

Jack L. Fry

Dean, College of Agriculture

Dean for Graduate Studies and Research